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SOY BEAN SEED-COAT, POTENTIAL RENEWABLE RAW-MATERIAL FOR ALCOHOL PRODUCTION

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SUMARY

The seed coat was shaken for different periods of time, from 12 hr to 96 hr in sterile distilled water pre-adjusted to pH 8. The contents of the flask filtered and pH adjusted to 4.6. Next the solution was heated for 20 min at 90^{0}C in a waterbath, filtered and media prepared from the filtrate. These media were inoculated with 10% volume of a strain of **Saccharomyces Cerevisiae**. *The suspension shaken on a rotary shaker at 250 rpm and 30^{0}C ± 1^{0}C for 48 hr after which the culture filtrate was distilled and the amount of alcohol measured according to the alcoholometric tables of the U.S. Pharmacopia. Thus up to 1.3% of alcohol could be obtained.*

Key words:
Soybean Seed Coat, Alcohol, Ethanol, Renewable feed stock.

1. INTRODUCTION

As the world resources of crude petroleum went on descreasing, research into various alternatives of cheap source of energy became more vigorous. As a result we have various forms of energy today. A close look at the presently available energy sources; however, reveals that except for the hydroelectricity, other methods of generating eletricity, namely thermal and nuclear; and other sources of energy, thermal, nuclear and solar are not as cheap as the liquid energy[9 10]

Thus began the quest for liquid energy source, but before long it was proved that the ethanol or liquid methane (biogas converted into methanol) are by far the cheapest sources of liquid energy. So embarked the era of research on various renewable feed-stocks (substrates) for the production of ethanol, which was rather cheap, safe and easy to produce. First trials were done with everknown substrate-sugar cane juice. Molasses was second. According to a recent international survey[9] today exist some half a dozen crop plants, which could be used as raw material for the production of ethanol. The list is still topped by sugar cane juice, next in the order being the sweet sorghum and cassava, with their respective merits and demerits.

The use of above named crop plants for the production of alcohol, however; posed two serious disadvantages. On one hand they compete with the available food or food comodity and on the other with the vast agricultural land which could be used for the production of such food.

A recent work[6] cites the use of husks and brans of many grains, like rice, wheat etc. for the manufacture of alcohol, although the economics and yield of alcohol from these sources are still to be evaluated. Thus soybean seed coat or husk also seems to be a good potential for Brazil because of the increasing (i) production of soy bean in the country which attained 15.1 x 10^6 metric tonnes in 1980 and (ii) export of crude flour (farelo) from 295000 metric tons in 1969 to 6x10^6 tons in 1980[11]. Since, for the production of any kind of soy-products to be used for human consumption, the first industrial step is the separation of the seed-coat[11], as a consequence of the increased demand of soy-products, there simply will be an increased availability of this residue. Presently this residue is an industrial effluent. Although review of literature does not reveal any information on the actual composition of soy-bean seed coat, the grain soy-bean has about 30% carbohydrates[1,2,5]. The water-insolubule carbohydrates of soy-bean are composed of cellulose and hemicellulose, which can be hydrolyzed by acid or/and enzyme[4].

Utilizing soy-bean milling residue, some results have already been reported from this laboratory[7]. Present report is on the use of soy-bean seed-coat for the production of ethanol.

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MATERIAL AND METHODS

Preparations of soy-bean seed coat extract: Using a rotary shaker, ten gram samples of soy seed coat contained in a 500 ml erlenmeyer flask were shaken at 25°C ± 1°C and 250 rpm for different periods of time, ranging from 12 hr to 96 hr in 150 ml of sterile distilled water pre-adjusted to pH 8 with 0.5% $NaHCO_3$. After the designated period, the contents of the flask were filtered and pH adjusted to 4.6 with 1N HCL. Next the solution was heated for 20 min at 90°C in a water bath to precipitate the proteins inherent in the soy-bean seed coat, and also to give coccion. Finally the extract was cooled, filtered and culture media prepared from the filtrate. Depending on the duration of trituration of seed coat of soy-bean, the extracts were designated as M12, M24, M36, M48, M72 & M96.

The organism – **Saccharomyces cerevisiae**: α ade 1 obtained as a gift from Dr. J. F. T. Spencer of Thames Polytechnic, London, England, U. K. , was used for all the studies reported here.

Maintenance of stock culture: The yeast was grown on slants of sabouraud dextroxe agar (SDA) for 48 hr at 28°C, and stocked at 4° until use.

Preparation of inoculum: The inocula were prepared by shaking for 24 hr at 28°C and 250 rpm a loopful of above stock culture in 100 ml of YPG medium contained in an erlenmeyer flask of 250 ml capacity.

Culture media: For the preparation of inocula: yeast extract, peptone, glucose broth (YPG) was used as described earlier [7].

For screening studies on the production of alcohol:

To all the extracts of soybean seed coat was added the mineral salts solution (MSS) containing g/l sodium nitrate, 2.0; monobasic potassium phosphate, 1.5; magnesium sulphate; 0.5 potassium chloride, 0.5 and ammonium sulphate, 2.0. Each of the extracts prepared by trituration of soy-bean seed coat for any given lenght of period, were devided into six groups. The first of which contained 0.2% of peptone only and designated A; second B–containing, 0,2% yeast extract only; 3rd C–0.1% yeast extract, 0.2% peptone and 1.5% glucose; 4th called–D with 0.75% peptone and 0.1% yeast extract; 5th as E 0.2% peptone and 0.10% yeast extract and the sixth, F without any supplementation of peptone, yeast extract or glucose (Table – 1). The pH of the each of the un-autoclaved media was preadjusted to 5.0 with 1N HCl. The pH remained unchanged after sterilization. These media were used to study the effect of culture medium on the alcohol yield.

Alcoholic fermentation: All the media were inoculated with a 24 hr culture of *S. cerevisiae* strain α ade 1 in a final concentration of 1/10 volume of the culture medium, the flasks shaken at 250 rpm and 28 ± 1°C for 48 hr. The fermentation was carried out in 125 ml erlenmeyer flasks containing 25 ml of the must.

Determination of alcohol: after 48hr, the culture was centrifuged at 5000 x G for 15 min, the supernatant distilled and quantity of alcohol assayed using density measurements and alcoholometric tables of United States Pharmacopia XIX[8].

RESULTS AND DISCUSSION

Results obtained with in the experimental conditions (Table 2) very clearly showed that higher amount of alcohol was obtained only in the treatments where the extract was prepared by shaking the seed coat for 12hr and 96 hr. From the yields of alcohol obtained it is very clear that when the concentration of both peptone and yeast extract was high i. e. media C and D (Table 2), the yield of alcohol was diminished. It was also interesting to note that in all the treatments, next best yield of alcohol was obtained, in media E.

Similar results were obtained with the soy-bean milling residue by us[7]. When the extract was not supplemented with glucose, the production of alcohol was highest, although higher alcohol yield was also obtained with lower concentrations of organic nitrogen (Table 1).

The lower yields of alcohol could be explained on several bases. The first of them could be the catabolite repression in media supplemented with glucose, an universally known physiological phenomenon of yeast metabolism. One could argue as to the concentration of glucose that was present in the media, as too little to cause catabolite repression. Nevertheless considering the amount of glucose that would have been available from the soy-bean seed coat, the concentration could be high enough to cause catabolite repression for the organism used. Another limiting factor could be the presence of tetra and trisacharides and the cellulose which would not have given enough concentration of glucose for continued production of alcohol. Yet another factor could be the intolerance of the yeast used to the alcohol concentration. Finally it has been shown that higher concentrations of nitrogen also have inhibitory effect on the production of alcohol[2]. Thus the lower yield of alcohol demonstrated in present work could be attributed to an individual or composite effect of the above stated factors.

The possible explanations for higher pH of spent media which did not give better yield of alcohol could be attributed to the[1] production of fusel oils[11] degree of extraction of carbohydrates and other constituents of soy bean coat as a result of the trituration.

In conclusion, although the optimal conditions for using the seed coat of soy-bean as a substrate for production of alcohol were not well predicted, based on experimental results herein obtained, one could suggest that this agricultural residue has a promising future as a raw material for the production of alcohol.

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TABLE 1: Composition of various media prepared from the soy bean seed coat extract to study the effect of media component on the alcohol production.

MEDIA	Quantities (in g/L) of		
	glucose	peptone	yeast extract
M12A, M24A, M36A, M48A, M72A, M96A	0.0	2.0	0.0
M12B, M24B, M36B, M48B, M72B, M96B	0.0	0.0	2.0
M12C, M24C, M36C, M48C, M72C, M96C	15.0	5.0	1.0
M12D, M24D, M36D, M48D, M72D, M96D	0.0	7.5	1.0
M12E, M24E, M36E, M48E, M72E, M96E	0.0	2.0	1.0
M12F, M24F, M36F, M48F, M72F, M96F	0.0	0.0	0.0

TABLE 2: Effect of medium composition on the final pH and the alcohol production by *Saccharomyces cerevisiae* strain α ade 1 in media prepared from the extracts of soybean seed coat.

Medium	Final pH[a]	Alcohol yield[b] (in %)	Medium	Final pH[a]	Alcohol yield[b] (in %)
M12A	5.10	0,80	M48A	5.93	0.40
M12B	5.12	0.80	M48B	5.84	0.50
M12C	5.45	0.90	M48C	5.84	0.50
M12D	5.33	0.90	M47D	5.83	0.40
M12E	5.58	1.30	M48E	6.85	0.70
M12F	5.06	1.00	M48F	5.85	0.60
M24A	5.22	0.40	M72A	5.87	0.40
M24B	5.81	0.50	M72B	5.81	0.50
M24C	5.83	0.40	M72C	5.86	0.50
M24D	5.85	0.40	M72D	5.80	0.40
M24E	5.90	0.80	M72E	5.96	0.65
M24F	5.80	0.60	M72F	5.92	0.55
M36A	5.87	0.40	M96A	5.36	0.70
M36B	5.87	0.50	M96B	5.52	0.70
M36C	5.96	0.50	M96C	5.65	0.60
M36D	5.82	0.40	M96D	5.55	0.80
M36E	5.93	0.60	M96E	5.38	1.10
M36F	5.89	0.50	M96F	4.70	1.00

a. The values are average of the readings taken of duplicate replicates at the end of experimental period.
b Average of two replicate determinations carried out by specific gravity measurements.

RESUMO

Neste trabalho são apresentados os resultados sobre obtenção de álcool etílico a partir de casca de soja. A casca foi agitada por diferentes períodos de tempo de 12h a 96h em água destilada esterelizada, pré-ajustada para pH 8. Os conteúdos do frasco foram filtrados, o pH ajustado para 4,6; os conteúdos cozidos, esfriados e filtrados. Os filtrados com ou sem suplementação com extrato de levedura, peptona e glicose em conjunto ou separadamente foram usados como mosto para fermentação por S. cerevisiae. *As suspensões foram agitadas a 250 rpm e 30°C ± 1°C 48h, e após este período, a quantidade de álcool calculada de acordo com a tabela alcoolométrica da Farmacopia dos Estados Unidos, foi de até 1.3%.*

PALAVRAS-CHAVE:
Casca de soja, álcool; etanol, matéria-prima renovável.

REFERENCES

1. IDA, E. I.; DA SILVA, R. S. F. & RAO, C. S. Oligossacarídios da soja: Problemas e soluções. Arq. Biol. Tecnol., Curitiba, *24 (3)* : 461 – 467, 1981.

2 JONES, R. P. ; PAMMENT, N. & GREENFIELD, P. F. Alcohol fermentation by yeasts: The effect of environmental and other variables. Proc. Biochem., Watford, England, *16* :42 – 49, 1981.

3. KAWAMURA, S. & TODA, M. Isolation and determination of sugars from the cotyledon, hull and hypocotyl of soy-beans by column chromatography. Tec. Bul. Fac. Agr. Kagawa Univ. Japan, *18 (2)* : 138 – 141, 1967.

4. LYONS, T.P. Ethanol production in developed countries. Proc. Biochem., Watford, England, *18 (2)* : 18 – 25, 1983.

5. SMITH, A. K. & CIRCLE, S. I. Soybeans: Chemistry and Technology – West port, Avi Publishing Co., U. S. A. 1978, Vol. 1.

6. SRIVASTAVA, K. C. A Critical study on the use of agricultural by-products for the manufacture of alcohol. IIo. Seminário sobre uso de Biomassa em Indústria. FIJAN, IDEG. Rio de Janeiro, R J, BRAZIL, 1 – 29, 1982.

7. SRIVASTAVA, K. C; POPPER, I.O.P. & WOSIACKI, G. Preliminary studies on the utilization of soybean milling residue for the production of alcohol. Proc. IV th Int. Symp. Alcohol Fuel Tecnol., Guaruja, SP., Brazil, 987 – 990, 1980.

8. The United States Pharmacopia – United States Pharmacopial Convention, Rockville, Md., U. S. A. , 1974.

9. World Bank – Alcohol production from the biomass in the developing countries. Washington D. C. U. S. A., p. 69, 1980.

10. World Bank – Renewable energy resources in the developing countries. Washington D. C., U. S. A., p. 33, 1981.

11. ZANGELMI, A. C. B.; TAGLIOLATTO, M. A. , DIAS, E. L. & LANGE, D. A. Produtos de soja: leite, farinha e outros. Série Tecnologia Agro Industrial V. 10 p. 157. Coordenadoria da Indústria e Comércio da Secretaria da Indústria, Comércio, Ciência e Tecnologia. Estado de São Paulo, Brasil, 1982.