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Bioconversion of Distillery Sludge (Treated) to Lysine and its Biological Evaluation

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ABSTRACT

Distillery sludge (brewing waste) was enriched with lysine by using *Brevibacterium flavum* as fermentative organism. The sludge as washed thrice to reduce the soluble carbohydrates and minerals. It was washed by centrifugation before using it as fermentative substrate. Proximate analysis of native and washed distillery sludge was done. It was found that as the number of washings increased, protein contents increased (18.25 – 25%) and ash contents decreased (37.14 – 29%) up to three washings. Prior to the production of biomass protein, certain conditions like substrate water ratio (40%), fermentation period (48h), effect of urea (1%), molasses (6%). Corn steep liquor (3%) and C:N (7.25:1) were optimized. These conditions were then applied to produce biomass protein from washed distillery sludge on large scale. The biomass thus produced contained 37.4%, crude protein, 24.06% true protein, 0.66% ether extract, 25% ash, 189 mg/100ml lysine and 2.87% RNA content. Biomass was then subjected to amino acid analysis which revealed presence of 12 amino acids. The chemical score of protein was 0.909 and limiting amino acid was methionine. The protein quality of biomass was tested in terms of digestibility, which showed an average digestibility of 90.47%.

Key Words: Bioconversion; Distillery sludge; Lysine

INTRODUCTION

With the outset of exploding population, the traditional source of protein and energy are no longer appreciable. Cereals which were thought to be the major nutritive element for both biomass and livestock lack good quality of protein and essential amino acid like lysine, arginine and threonine (Saima, 1996). Brewing industry is one of the oldest and largest industries in the world in which *Saccharomyces cerevisiae* is good for alcoholic fermentation. In Pakistan, the total yeast cell mass produced by the brewing industry in the form of distillery sludge is estimated to be 16,000 tons/year. Distillery sludge is the by-product of brewing industry, which is not being utilized properly. This distillery sludge has a great potential in itself. It contains a substantial quantity of protein but limiting in lysine (Basit, 1996). Thus, it is essential to enrich it with lysine before supplementation in poultry and livestock rations.

A microbial process for lysine production was first developed by a combination of diaminopimetlate production by lysine auxotroph of *E. coli* and decarboxylation of compound by *Aerobactor aerogens.* Cane molasses is not generally used as carbon source in the industrial production of lysine, although other carbohydrate material, acetic acid and ethanol can be used (Reed, 1987). A project was carried out to develop a fermentation process by utilizing washed distillery sludge as a substrate for the production of lysine enriched biomass by *Brevibacterium flavum*. The biomass thus produced was chemically and biologically evaluated.

MATERIALS AND METHODS

The distillery sludge procured from Shakhargung Sugar Mills Ltd., Jhang was washed to reduce the soluble carbohydrates and minerals. Washed sludge was obtained by centrifugation. Samples of washed and unwashed sludge were analysed for its chemical composition, before fermentation with *B. flavum*. Its enrichment with lysine was achieved by fermenting it under optimum conditions. Culture conditions were optimized with respect to varying substrate water ratios, fermentation periods, addition of urea, molasses, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, KH_2PO_4, corn steep liquor and C:N ratio. Biomass produced under optimum conditions was dried at 70°C under vacuum. The dried biomass was analysed for its proximate composition (A.O.A.C., 1984), true protein and RNA content (Ceriotti, 1955), amino acid profile (Moore & Stein, 1954), lysine content (Chaves *et al.*, 1988). The protein quality of the biomass was tested in terms of digestibility using three enzyme method (Pederson & Eggum, 1981). The data obtained was analysed according to CRD and it was subjected to statistical analysis by constructing ANOVA table and significance of differences between means were compared by DMR test (Steel & Torrie, 1992).

RESULTS AND DISCUSSION

The main objective of the study was to upgrade the washed distillery sludge with respect to lysine content. It was achieved through fermentation of the washed

distillery sludge with *B. flavum*. The lysine content was increased from 0.54 to 4.78 g/100g. The quality of biomass was evaluated by *in vitro* digestibility trials using three proteolytic enzymes.

A. Chemical composition of sludge and biomass: As the number of washings increased, the moisture content increased as compared to each other but decreased in comparison to the native form of the substrate. Ash content decreased as the number of washings increased and also in comparison to native form of substrate but the carbon content was found to be decreased (Table I).

Table I. Proximate composition of distillery sludge and biomass

Comp. (%)	Active sludge	1 time washed	2 time washed	3 time washed	Bio-Mass
DM	27.00	42.10	40.76	38.02	21.25
Ash	37.00	35.00	32.00	29.00	25.50
EE	1.10	1.00	1.00	1.00	066
CF	0.00	0.00	0.00	0.00	0.00
CP	18.59	20.78	23.00	25.15	37.18
NFE	43.31	43.22	44.00	44.85	37.16
TP	13.12	15.31	17.50	19.69	24.06
RNA	1.44	1.61	1.78	1.94	2.88
CC	42.40	-	-	30.00	-
Moisture	75.50	57.90	59.24	61.98	78.75

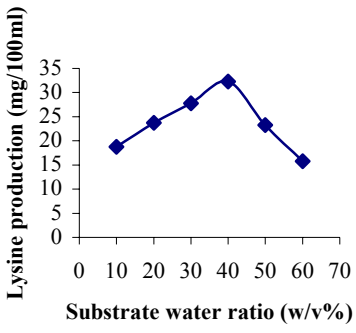
Comp.= Component; DM=Dry matter; EE=Ether extract; CF=Crude fibre; CP=Crude protein; TP= True protein; CC= Carbon content

Ash content of the biomass decreased, as the substrate was used in the fermentation medium in small quantity. The crude protein, true protein and RNA content of biomass was also increased.

B. Optimum conditions: The following conditions were established for the production of protein biomass enriched with lysine.

Substrate water ratio. There was gradual increase in the production of lysine from 10 to 40% substrate which decreased with higher substrate water ratio (Fig.1).

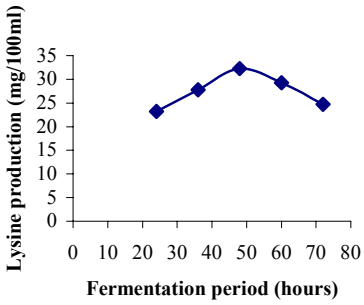
Fig. 1. Effect of various substrate water ratio on lysine production



Results are supported by Naz (1998), Sattar (1998) and Yawar (1998) who optimized the distillery sludge at 40% substrate water ratio. Although substrate used in present study was completely washed.

Fermentation period. The production of lysine increased from 24 to 48 h, which decreased with further increase in time period (Fig.2). Results supported the

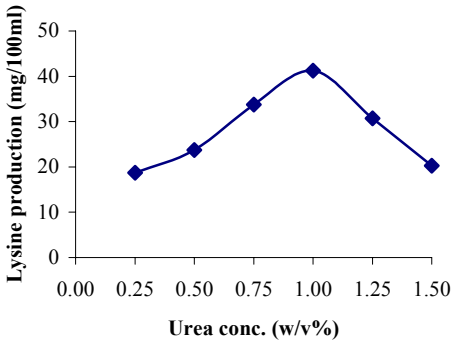
Fig. 2. Effect of time periods on lysine production



findings of Saleem (1997) who observed maximum protein production after 48h of incubation.

Addition of urea. Addition of urea resulted in production of lysine from 0.5 to 1.0%. It was found decreased with higher urea concentration (Fig.3). The findings are in line with those of Li *et al*. (1987) who reported an increase in lysine yield by addition of urea.

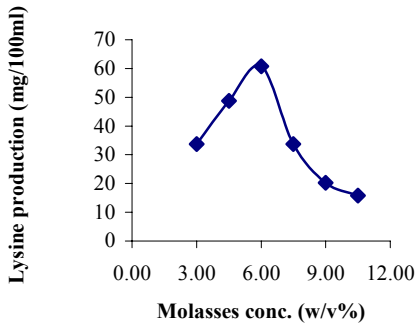
Fig.3. Effect of various urea concentrations on lysine production



Addition of molasses. The production of lysine increased with an addition of 3 to 6% molasses. It decreased with further higher molasses concentration (Fig.4). Results of the present study are not in line with Naz (1998) and Sattar (1998) who got maximum production of lysine in the presence of 1% molasses in the fermentation medium.

The reason with 6% molasses for maximum lysine in the present study is use of completely washed sludge. However, Li *et al.* (1987), and Plachy and Ulbert (1989)

Fig. 4. Effect of various concentrations of molasses on lysine production



used 10 and 25% molasses, respectively for the production of maximum lysine.

Ionic concentrations. For optimizing of ionic concentration for the growth medium to produce maximum lysine, three different ionic concentrations i.e. $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4 were used. Addition of $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and KH_2PO_4 showed negative effect on lysine production (Fig.5,6 & 7) because the substrate and fermentation medium already had enough quantity of these minerals for the maximum production of lysine. So these were not required for the production of lysine and hence in these case control was considered as optimum for maximum production of lysine.

Addition of CSL. There was initial increase in the production of lysine from 1 to 3% which decreased thereafter (Fig. 8). Results can be compared with those of Yawar (1998) and Naz (1998) who used 0.75 and 5% CSL, respectively for maximum production of lysine. The difference in results is due to change in substrate and fermentation period from Yawar (1998).

Carbon Nitrogen Ratio. In the present study, C:N is maintained to get the maximum biomass protein which is also be supported by Hashmi *et al.* (1989) who optimized the C:N 25:1, 12.5:1 and 25:1, respectively for maximum production of biomass protein.

Biological evaluation. The average digestibility of microbial protein by three enzyme method *in vitro* was found to be 90.4%. The findings are in line with Enriquez and Rodriquez (1983) who observed nitrogen digestibility of 90% from biomass obtained from bagasse when fed to rats. However, Setala *et al*. (1984) also observed average digestibility of 80.70% for microbial protein in pepsin-HCl and Trpsin-Chymotrypsin solution.

Amino acid profile. Amino acid profile study showed that the biomass contained twelve amino acid (Table II & III). The chemical score of the protein isolate was 0.909%, methionine was the first limiting amino acid and the second limiting amino acid was phenyl alanine. The lysine content in amino acid profile was 4.78/100g.

Fig. 5. Effect of various concentrations of $CaCl_2$ On lysine production

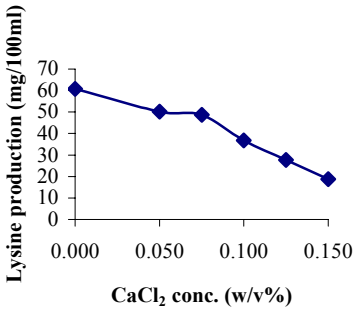


Fig. 6. Effect of various concentrations of $MgSO_4.7H_2O$ on lysine production

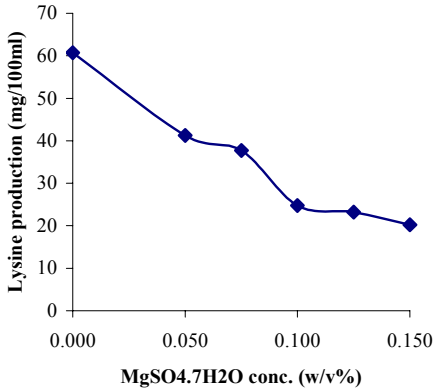


Fig.6 Effect of various conc. of MgSO4.7H2O on the production of lysine

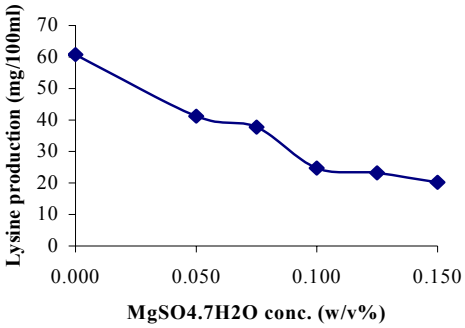


Fig. 8. Effect of various concentrations of liquor on lysine production

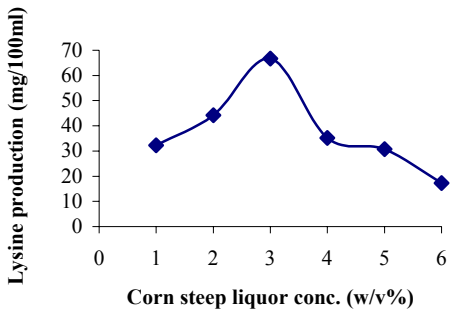


Table II. Amino acid profile of biomass produced using *Berevibacterium flavum*

S.No.	Amino acid	Percent at protein basis (g/100g)
1.	Aspartic acid	3.990
2.	Valine	0.506
3.	Serine	1.780
4.	Glysine	1.980
5.	Alanine	0.085
6.	Leucine	1.000
7.	Arginine	0.780
8.	Isoleucine	2.724
9.	Phenylalanine	0.072
10.	Lysine	4.780
11.	Methionine	0.020
12.	Threonine	1.780

Table III. Chemical score of biomass protein using FAO (1957) method

Amino acid	FAO amino acids pattern (mg/g)	Biomass amino acid (mg/g)	Available amino acid (%)
Isoleucine	42.0	27.24	64.85
Leucine	48.0	10.00	20.83
Lysine	42.0	47.80	113.80
Methionine	22.0	0.20	0.91
Phenylalanine	28.0	0.92	3.27
Threonine	28.0	17.82	63.64
Valine	42.0	5.06	12.05

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