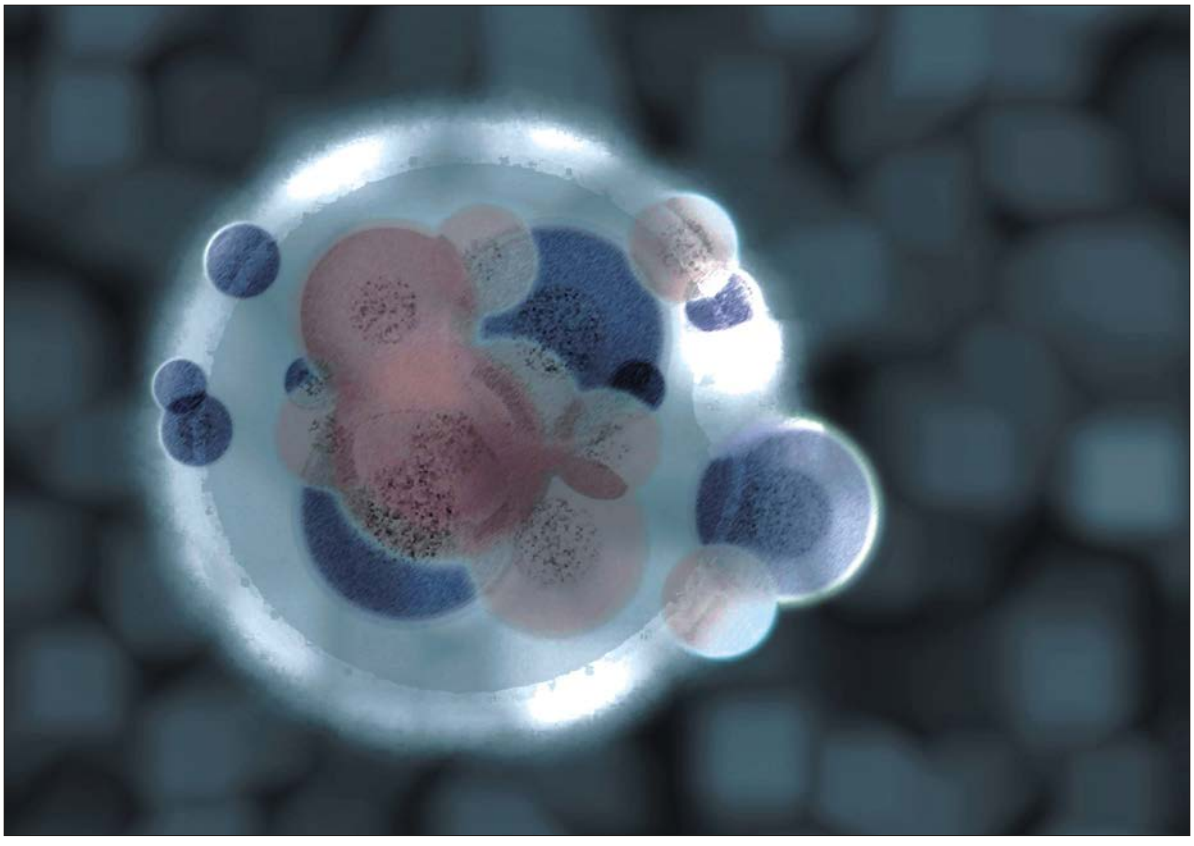

Journal of Biological Sciences

ISSN 1727-3048

science alert

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OnLine Journal of Biological Sciences 1 (6): 507-510, 2001

Effect of Physiochemical Treatments on *Brevibacterium flavum* for Production of Lysine

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Abstract: *Brevibacterium flavum* maintained at 7.0 pH level and at 37°C temperature, gives maximum production of lysine. When the organism was given UV shocks for 30 minutes, gave the lysine in maximum quality. Water substrate ratio, addition of molasses, addition of corn steep liquor were optimized. At 40% water substrate ratio, addition of 4% corn steep liquor and 4% molasses bacteria was most efficient in the production of lysine. The biomass thus produced contained 32.8% crude protein, 20.87% true protein, 34% ash and 1.1% fat contents on the basis of dry matter. There was no increase or decrease in ether extract and crude fiber. The biomass produced was analyzed by three-enzyme method. Its digestibility was 85.69% in single cell protein biomass. Amino acid profile showed that lysine production was 21.48mg/g on the basis of protein.

Key words: Molasses, lysine, *Brevibacterium flavum,* physiochemical treatments

Introduction

Much research activity has been focussed on microbial production of amino acid. With the hope of improving the nutritional value of low cost vegetable proteins by enrichment with essential amino acids. Amino acids have extensive industrial applications. About 66% of the amino acids produced are used in food industry 31% as feed additives, 4% in medicine and cosmetics and as a starting materials in the chemical industry. Lysine is added to bread in Japan and in some countries soy products are enhanced by the addition of methionine (Crueger and Crueger, 1990).

In Pakistan total yeast cell mass produced by the sugar industry, in the form of distillery sludge is estimated to be 16000 tons/year. It contains protein but limiting in lysine (Basit, 1996).

Enrichment of this distillery sludge with lysine was done by fermentating it with *B. flavum* under optimum conditions. The *B. flavum* was treated with temperature, pH and ultraviolet shocks for maximum lysine production. A plan was developed to treat *B. flavum* through physio-chemical changes for increased lysine production, when cultured on distillery sludge and biomass was produced, analyzed chemically and biologically.

Materials and Methods

Source: To produce lysine-enrich biomass using distillery sludge as substrate, fermented with *B. flavum*, distillery sludge was procured from Shakargung Sugar Mills, Jhang. Proximate composition of distillery sludge was determined by methods described by AOAC (1984) (Table 1).

Organism: To investigate the production of biomass from distillery sludge, the fermentative organism *B. flavum* was received from the Department of Animal Nutrition, University of Agriculture, Faisalabad. The organism was maintained on nutrient agar slants (Reed, 1987). Fresh inoculum was prepared by aseptically transferring culture raised on the agar slants to an autoclaved 250ml conical flask containing 50ml of inoculum media (Table 2). The pH of the medium was adjusted at 7.00. The flask was incubated on an orbital shaker working at the rate of 100-120 rpm for 3 days at 37°C. Concentration of the organism was adjusted to an optical density of 0.6 at 610 nm by diluting the suspension with sterile distilled water to get the homogeneous suspension. This inoculum was used to investigate the optimum conditions of the growth medium for the production of lysine.

Table 1: Proximate analysis of distillery sludge on dry matter base

Components	%age composition	
	Before fermentation	After fermentation
Ether extract	1.10	1.10
Crude fiber	0.00	0.00
Crude protein	18.59	32.81
Ash	37.6	34.00
True protein	13.13	20.87

Table 2: Composition of nutrient agar slants and inoculum medium

Material	Agar medium g/100 ml	Inoculum Medium
Agar	3.0	-
Beef extract	0.5	0.5
Peptone	1.0	1.0
NaCl	0.5	0.5
Distilled water	100ml	100ml

Optimum conditions: The growth medium employed to culture the *Brevibacterium flavum* on distillery sludge for the production of lysine was developed (Chaves *et al*., 1988). Optimum conditions like substrate to water ratio, fermentation period, addition of molasses, addition of corn steep liquor were optimized. Then temperature, pH and UV shocks were given to the *B. flavum* to produce maximum lysine. After each experiment, the biomass produce was analyzed for lysine estimation.

Lysine estimation method: The lysine of the biomass was estimated by the method of Chaves *et al*. (1988). A standard curve was made by taking different volumes of standard solution of lysine. HCl (0.15g/100ml) in six test tubes while 7th tube served as blank. The different lysine HCl concentration as well as blank treated with the reagents is given in Table 3.

The test tubes were placed in cold water for one hour and were shaken after every five minutes. Then the volume was made 20 ml by adding distilled water. The absorbance of each standard was checked on spectrophotometer at 545 nm wavelength. The sample was also proceeded in the same way as the standard.

Table 3: Lysine estimation method

Reagents	Test tubes						
	1	2	3	4	5	6	7
Standard lysine	0.1	0.2	0.3	0.4	0.5	0.6	Blank
Distilled water	4.9	4.8	4.7	4.6	4.5	4.4	5.0
Sodium nitroprusside	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium tetraborate	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Place the test tube in cold water for three minutes then add the							
Acetone	4.0	4.0	4.0	4.0	4.0	4.0	4.0

Table 4: Amino acid profile of the biomass produced using *Brevibacterium flavum* as micro-organism

Amino acid	Percent at protein basis (g/100 g)
Aspartic acid	4.0457
Threonine	1.6525
Serine	1.5275
Glutamic acid	1.95975
Glysine	1.830
Alanine	0.0725
Isoleucine	2.449
Leucine	0.968
Phenylalanine	0.117
Lysine	2.148

Results and Discussion

Proximate analysis of biomass showed that crude protein increased from 13.13 to 21.87%. Ash contents of the biomass reduced to 4%. Amino acid profile showed that lysine production was 21.48mg/g on the basis of protein. There was no increase or decrease in ether extract and crude fiber. The proximate composition of the biomass is given in Table 1.

The different optimum conditions employed to enhance the production of lysine and result obtained are discussed below:

Substrate to water ratio: In order to know the suitable substrate to water ratio for the maximum production of lysine, various substrate to water ratio viz. 10, 20, 30, 40, 50 and 60 (v/v%) resulted in 48.75, 53.25, 56.25, 49.50 and 49.50 mg/100 ml lysine respectively. It was found that 40 (v/v%) substrate water ratio was the best of all the ratios tested as it resulted in maximum production of lysine 59.25% (Fig. 1).

Alam (1986) who obtained maximum crude protein contents of biomass from rice polishing when fermented with *Arachniotus* sp. In shake flask medium with 5% (w/v) substrate:water ratio after 72 hours of incubation at pH 4 and 37°C temperature.

Fermentation period: After optimizing the substrate to water ratio for the maximum production of lysine by *Brevibacterium flavum*, the incubation period at which maximum lysine produce was determined. The different fermentation periods which were tested are 24, 36, 48, 60 and 72 hours. The lysine production at these different incubation periods are shown in Fig. 2, which is 29.25, 45.75, 61.50, 54.00 and 36.75 mg/100 ml. It was found that lysine production is maximum at 48 hours of incubation.

Results are in line with Sana (1997) who improved the quantity and quality of protein contents of corn stover by fermenting with *Arachinotus* sp. She found that 8.20% crude protein was recovered after 48 hours of incubation.

Addition of molasses: Addition of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% molasses (cane) to the fermentation medium after 2 days of incubation resulted in 78.75, 83.25, 74.25, 69.75, 60 and 60 mg/100ml lysine. The results revealed a marked increase in lysine production in the growth medium containing 1% molasses (Fig. 3).

Khan *et al*. (1990) observed that addition of 5% molasses after 48 hours of incubation enhanced the total protein from 7.71 to 11.97 mg/ml.

The result of present study are substantiated by Lawford *et al*. (1979) who used can molasses as source of growth limiting carbon in the fermentation media for the production of single cell protein using *Candida utilis* NRRL Y-900. At one percent reducing sugar with the addition of zinc powder resulted in an increase in biomass productivity from 1.7 to 2.6 g/l/h with the growth yield of 0.55g dry biomass/g reducing sugar utilized at the maximum dilution rate. The yeast biomass contained 50-55% protein.

Addition of corn steep liquor: It was found that there was gradual decrease in lysine from 1-4% then it was decrease with high percentage of corn steep liquor. Addition of 1, 2, 3, 4, 5 and 6 corn steep liquor to the fermentation media after 2 days of incubation resulted in 63.75, 66.75, 69.75, 75.75, 70 and 60 mg/100ml lysine (Fig. 4).

The results of present study are in line with findings of Morio *et al*. (1978) who fermented methanol with *Candida* Moy-657 and recovered maximum biomass protein when CSL (1 g/l) was added in medium. El-Ashwah *et al*. (1978) also obtained 34.38% biomass protein from medium containing molasses, sweet potato and corn steep liquor. The optimized level of CSL reported by Hashmi *et al*. (1989), Khan *et al*. (1990) and Mehmood-ul-Hassan (1992) were 0.4, 1 and 0.8%, respectively supplied at 72.48 and 48 hours also supported our findings.

Effect of Various Temperature Treatments on *Brevibacterium flavum* for the Lysine Production: The study was undertaken to see the effect of different temperatures viz. 35, 45, 55 and 65°C on *Brevibacterium flavum* for the production of lysine. The lysine production is 84.75, 78.75, 74.25 and 63.75 mg/100 ml respectively. The average values are given in Fig. 5.

Effect of pH: In order to know the effect of different pH levels of fermentation media on *Brevibacterium flavum* for the maximum production of lysine at different pH levels viz., 6.0, 6.3, 6.6, 7.0, 7.3 and 7.6 resulted in 65.25, 71.25, 76.50, 83.50, 72.67 and 67.60mg/100ml lysine production respectively. *B. flavum* was maintained at 7.00 pH level, it gives maximum production of lysine so it is optimum pH for the growth of *B. flavum* (Fig. 6).

Ultraviolet shock: Duplicates slants of *Brevibacterium flavum* was given UV shocks for 5, 10, 15, 20, 25 and 30 minutes.

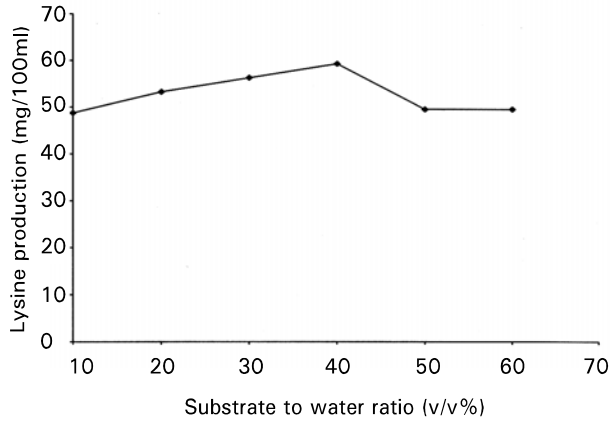

Fig. 1: Effect of various levels of substrate to water ratio on lysine production

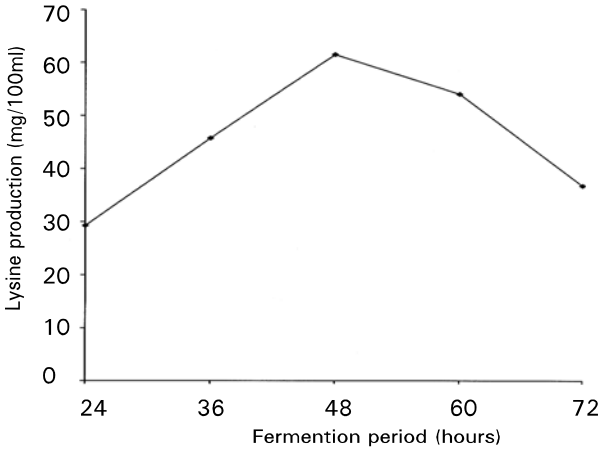

Fig. 2: Effect of various time period on lysine production

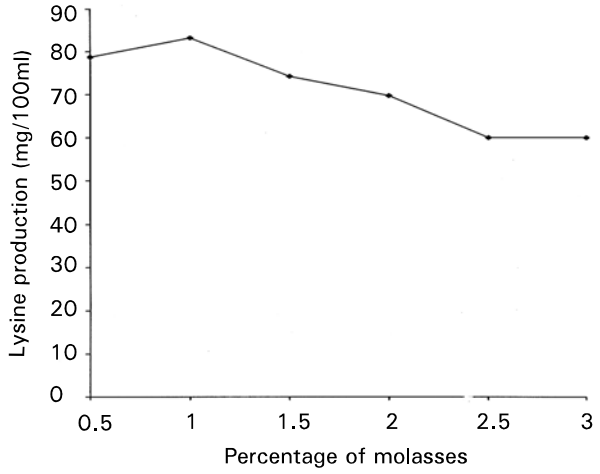

Fig. 3: Effect of various levels molasses on lysine production

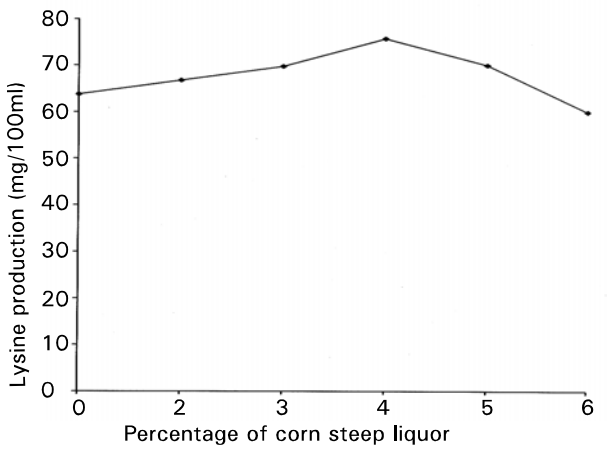

Fig. 4: Effect of various levels on corn steep liquor on lysine production

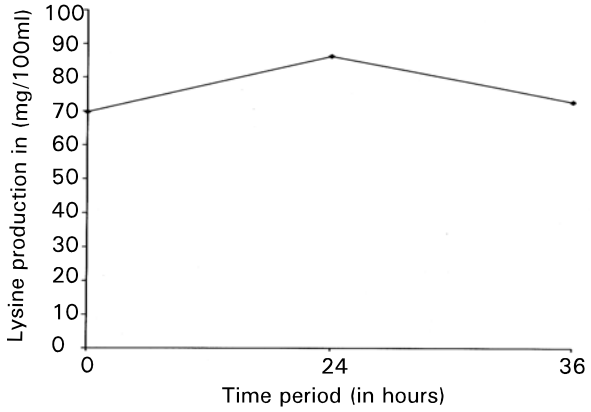

Fig. 5: Optimum period for temperature shocks on *Brevibacterium flavum* for the maximum production of lysine

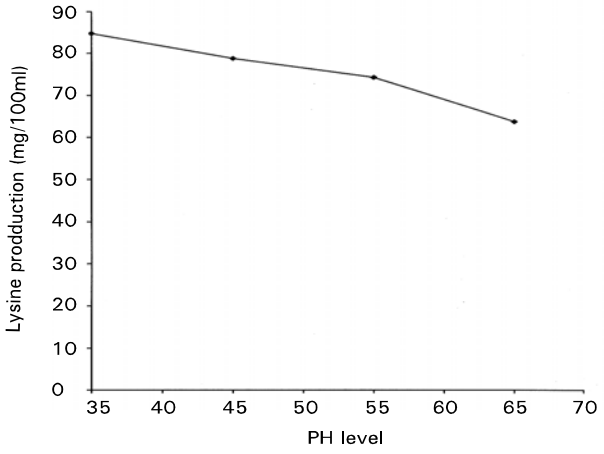

Fig. 6: Effect of diffrent levels of pH on Brevibacterium flavum for the production of lysine

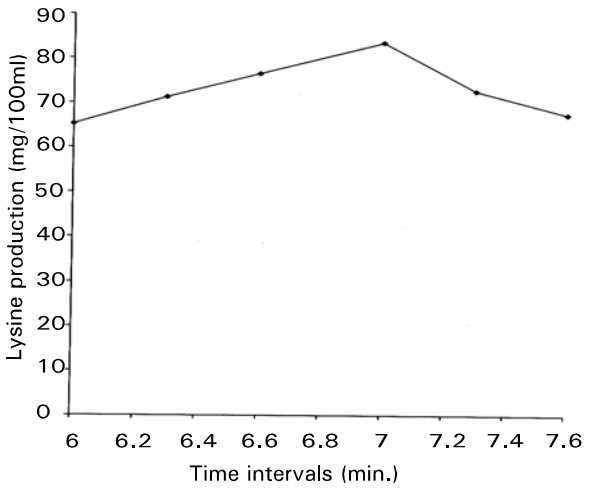

Fig. 7: Effect of ultra violet shocks on *Brevibacterium flavum* for the lysine production

The treated organisms give lysine 46.50, 55.50, 64.50, 65.00, 66.00 and 70.83 (mg/100 ml) respectively (Fig. 7). The result revealed an increase in the production of lysine, as the time interval for UV shocks was increased for 25 minutes but when the organism was kept for 30 minutes under ultraviolet light, the lysine production decreases at once which showed that when the *Brevibacterium flavum* was given more UV shocks, UV light inhibits its growth and lysine decreases. *Brevibacterium flavum* is not so much effective organism for the production of lysine. If any body wants to increase the lysine content of distillery sludge upto satisfactory extent, they must also concluded that the biomass obtained from sludge by fermenting with *Brevibacterium flavum* can be used as commercial source of some amino acids like aspartic acid and isoleucine and cannot be used as a better quality feed stuff for ruminants and human beings.

Biological evaluation: The average digestibility of microbial protein by three-enzyme method *in vitro* was observed. The digestibility was noted 85.69% in single cell protein biomass. The percentage digestibility is in acceptance region but not excellent.

The findings are in line with Johnson and Romillard (1983) who also reported 86.5% digestion coefficient of crude protein when brewer single cell protein was fed to growing weather sheep. However, Enriquez and Rodriguez (1983) observed nitrogen digestibility of 90% from biomass obtained from bagasse when fed to rats.

Amino acids: The biomass was analyzed for its amino acid profile presence of which revea. The amino acid composition is shown in Table 4. It was observed that the microbial biomass protein had some increase in amount of all amino acids especially the essential one. The chemical score of the protein isolate was 1.168. Methionine is the first limiting amino acid, while the 2nd limiting amino acid is phenylalanine. The results of the present study were in agreement with the findings of Athar *et al*. (1995). An overall increase in quantity of all amino acids was reported especially the lysine, glutamic acid and methionine along with increase in protein contents. The results are also comparable with that of Mehmood-ul-Hassan (1992) who studied the amino acid profile of beet pulp which was limiting in lysine. But the examined that all other amino acids were present in sufficient amount.

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