UTILIZATION OF DAIRY WASTE FOR BIOMASS PRODUCTION OF SINGLE CELL PROTEIN THROUGH SUBMERGED FERMENTATION

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ABSTRACT: The alarming rate of population growth has increased the demand for food production in third world countries leading to a yawning gap in demand and supply. This situation has created a demand for the formulation of innovative and alternative proteinaceous food sources. The production of single cell protein (SCP) is a major step in this direction. SCP is the protein extracted from cultivated microbial biomass. In present investigation, a simple process for utilizing whey protein and converting lactose to SCP in order to obtain products which enhance the biological value of microorganisms i.e., yeast, fungi and bacteria. These microorganisms were screened for their growth and SCP production using whey as a substrate. This study emphasizes on the selected species of organisms proved to be most suitable for production of microbial biomass and protein production on whey. The cultivation was carried out at 30°C on rotary shaker 150 rpm. The best results were obtained with yeast and fungi regarding biomass production. The utilization of whey as protein substrate bacteria was found to be more efficient than fungi and yeast. However for carbohydrates utilization from whey, yeast and bacteria were effective than fungi. The results achieved in all experiments showed that the selected species of organisms could be successfully used for SCP production by using whey as a substrate.

Keywords: *Single cell protein, Whey, Submerged fermentation, Dairy wastes.*

INTRODUCTION

Use of microbes as a food source may appear to be unacceptable to some people but the idea of consumption of microbes as food for man and animals is certainly innovative to solve the global food problem. For thousands of years man has been consuming, either intentionally or unintentionally, products such as alcoholic beverages, cheese, yogurt, soya sauce, and along with these products, the biomass responsible for their production (Tuse, 1984). The first written record of the utilization of microbes dates back to 2600 BC in Babylonia, as traces of bread were found there. By the time of Hammurabi, who ruled during the 12th century BC, baking had developed into a special craft. The discovery of leavened bread is generally attributed to the Egyptians (Jacob, 1944). Even today there are reports regarding the use of microbes in food and feed from many parts of the world (Singh, 1998). Currently SCP is produced from many species of microorganisms. These include algae, fungi and bacteria. It is convenient to use fungi and bacteria for production of SCP when grown on inexpensive waste material. Their rapid growth and high protein content have made them the prime candidates for use as sources of SCP. Several species of algae that are currently being used are cultivated on aquatic media (Tuse, 1984).

The world production of dairy products has reached 11–12 $\times$ 10^6 tons per year. The total amount of liquid whey produced in these processes is cca 10^8 tons. There are two kinds of whey; sweet and acid. This valuable by-product of dairy products contains many of the nutrients from milk and can be almost completely utilized for different (ethanol, yeast biomass, lactic acid) purposes. Whey is a liquid byproduct of the cheese making process that contains most of the water soluble components, and water, present in milk (approximately 5% lactose, 0.9% nitrogenous compounds, 0.8% minerals and small amounts of vitamins). The dairy industry must, therefore, try to attain a position where handling the whey does not prevent the industry from meeting the market demand for its products. Since, lactose in cheese whey is the major contributor to BOD, using the whey as a substrate for the production of SCP may reduce its pollution potential while results in the production of a value added product. The term SCP refers to dried cells

of microorganisms such as algae, actinomycetes, bacteria, yeast, molds, and higher fungi grown in large-scale culture systems for use as protein source in human food or animal feed. The most important characteristic of these single-celled organisms is their high protein content, ranging from about 40% to 80% of their dry weight on a crude protein basis. Also, their protein tends to be of high quality, more closely resembling animal protein than plant protein, and is generally readily available nutritionally.

The main aim of this study was to investigate the effectiveness of submerged fermentation of the yeast in cheese whey for the production of SCP.

MATERIALS AND METHODS

Organisms: The organisms, bacteria, yeast and fungi were selected in the present studies. Bacillus species and yeast were taken from the Food and Biotechnology and Research center (FBRC) PCSIR laboratories complex Lahore while *Aspergillus* species were obtained from Fungal Culture Bank, Institute of Mycology and Plant Pathology, University of the Punjab Lahore.

Media: The Yeast glucose agar medium for yeast, L.B medium for bacteria and MEA for fungi were used in the present studies.

Revival and maintenance of cultures: 100 ml of media with respect to organism was dissolved in 250 ml conical flask and cooked for 15 minutes. Media was autoclaved at 15psi (121°C) for 20 minutes and allowed to cool but not solidify. Media solution was poured in sterilized Petri plates and allowed to solidify. The plates were inoculated with a bit of inoculum of the required organism from the parent culture with sterilized inoculating needle and incubated at 25±2 °C for 7 days. The pH of the medium was adjusted at 5.0 with 1N HCl/NaOH

Preparation of slants: 100 ml of media with respect to organism was dissolved and cooked for 15 minutes and then transferred in to the test tubes. The test tubes were autoclaved at 15psi (121°C) for 20 minutes and then placed in tilted position for solidification. The slants were inoculated with organism from plates. The culture was revived and purified by repeated inoculation of plates and slants and stored at 4 ^{0}C.

Whey: Powder whey was obtained from market, while liquid whey was obtained from Halla Milk Factory Walton Road Lahore. It was transferred to 1 ½ litre plastic bottles. These bottles were stored in freezer at -25 °C until required. Prior to placing the whey into shake flask it was allowed to completely thaw at room temperature for 2-3 hours. Then whey was pasteurized at 60 °C for 30 minutes. It was then cooled to 0 °C for 30 minutes and latter on stand at room temperature for 24 hours. The process of heating, cooling and standing at room temperature was repeated thrice to destroy any vegetative or spore cells present in whey. Powder whey was sterilized and then allowed to dry completely at room temperature.

Inoculum Preparation: The inoculum of yeast, fungi and bacteria were streaked on YGA, MEA and LB media. The slants were placed in controlled environment and incubated at 25 °C for yeast, 37 °C for fungi and bacteria. The growth was observed after 48 hours. The yeast, fungal and bacterium inoculum was shifted in 250 ml flask having 50 ml pasteurized whey. For each organism, three replicates were used. The flasks were capped with non-absorbent cotton plug and mounted on the rotatory shaker which was set at 150 rpm. Before transferring the organism, the protein and carbohydrates contents of whey were also determined by spectrophotometeric method.

Culture conditions: Growth temperature was set at 30 °C under shaking condition for 4 and 6 days in case of yeast and fungi respectively and for bacteria for 2 days.

BIOMASS DETERMINATION

Fungi: In submerged fermentation method biomass is generally recovered by filtration or centrifugation. To quantify this biomass of a sample, it is gradually filtered through a pre- weigh filter paper. From the culture broth, the mycelium were filtered on filter paper and washed twice with distilled water. Then the net weight was measured

Yeast and bacteria: The cells were harvested through centrifugation, when centrifuged at 13000 rpm for 10 minutes in centrifuge tube. The supernatant discarded while pellet was washed twice with distilled water and lypholized for 15-20 hours at -50 °C.

Estimation of protein: The protein of substrate was estimated by Lowery et al (1951). It was used to estimate protein with bovine serum album as the standard.

Estimation of Carbohydrates: The estimation of carbohydrates was done by Phenol Sulphuric Acid method (Dubois et al., 1956).

STATISTICAL ANALYSIS

Treatment means, standard error and Duncan's Multiple Range Test (Steel et al., 1996) were calculated from the data obtained for various parameters using software package Costat version 3.03.

RESULTS AND DISCUSSION

Determination of SCP Biomass: The biomass of organisms grown in liquid whey and powder whey dissolved in 50 ml distill water was determined (Table 2). The fungi were separated from the liquid whey media by filtration while bacteria and yeast were separated from the whey growth media by centrifugation at high speed 13000 rpm for 10 min at 4°C. The weight of fungal biomass in liquid whey was found to be highest i.e., 0.9 g and 0.92 g for *Aspergillus niger* and *A. flavus* while least biomass observed in liquid whey in case of bacteria which was about 0.5g per 50 ml of liquid whey (Table 2). The biomass was recorded during the present study for all organisms by keeping them at room temperature and finally at 120 °C for 2-3 hrs. The maximum weight was obtained in powder whey in case of yeast (0.17g), but in bacteria least biomass was in range of 0.08-0.11 g while in fungi the biomass was recorded as 0.11-0.13 g. Therefore, Table 2 indicates that the maximum biomass in liquid whey was observed in case of fungi while maximum biomass in powder whey was observed in bacteria.

Estimation of Protein in Liquid and Powder Whey: The protein amount in both liquid and powder whey was determined by spectrophotometeric method at two stages. At first stage the protein contents were measured before adding the organism in the respective whey media while at second stage the protein contents were estimated after removing the organism from whey media. After specific duration of growth of organisms were grown comparatively for longer period in both type of whey media i.e. 3-4 days while for bacterial growth for 2 days were sufficient for proper growth. The liquid whey was sterilized by passing through 0.2µ filter while powder whey were also sterilized in the same way after dissolving the fixed quantity 200 mg in distill water. For liquid whey initial protein contents before adding organism were found to be 350 mg , 390 mg and 370 mg per 50 ml for bacteria, yeast and fungi while in case of powder whey dissolved in H_2O, the initial protein contents were found to be 200 mg per 50 ml of whey (Table 3).The maximum %age of protein was utilized by bacteria and yeast spp. i.e., 75% and 73% respectively, However in case of both yeast and fungi almost 50% of the total whey protein in liquid whey was utilized in growth. Least protein content of whey was utilized by *Aspergillus niger* in case of powder whey dissolved in distill water, similar pattern of protein utilization by organisms was observed as in case of liquid whey medium. The maximum utilization of protein content of powder whey medium was observed in bacteria; *Bacillus subtilis*, *Bacillus* sp.1, while for yeast and fungi almost 50% protein content were utilized by organisms. Table 3indicate that the bacteria are most efficient consumer of whey protein as compared to yeast and fungi however later two groups of organism showed almost same behavior regarding the utilization of protein contents from both liquid whey and powder whey dissolved in distill water.

Table 1: Growth of microorganisms (SCP) on dairy waste (whey)

a- Bacteria	
	Bacillus subtilis, *Bacillus* sp.1, *Bacillus* sp.2,
b- Yeasts	
	Candida sp, Saccharomyces sp.
c- Fungi	
	Aspergillus flavus, Aspergillus niger.

Table:2. Biomass determination of single cell protein

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Organism	Liquid whey				Powder whey			
	Biomass (g)				Biomass (g)			
	W1	W2	W3	Mean	D1	D2	D3	Mean
Bacteria								
Bacillus subtilis	0.51	0.49	0.52	0.50 ±0.01	0.11	0.09	0.10	0.1 ±0.02
Bacillus sp1	0.46	0.46	0.51	0.47 ±0.01	0.07	0.07	0.08	0.07 ±0.03
Bacillus sp2	0.63	0.52	0.51	0.55 ±0.03	0.13	0.11	0.11	0.11 ±0.01
Yeast								
Candida sp	0.71	0.73	0.73	0.72 ±0.01	0.14	0.15	0.17	0.15 ±0.01
Saccharomyces sp	0.78	0.76	0.71	0.75 ±0.01	0.12	0.12	0.16	0.13 ±0.03
Fungi								
Aspergillus flavus	0.86	0.81	0.91	0.86 ±0.02	0.14	0.10	0.11	0.11 ±0.02
Aspergillus niger	0.83	0.82	0.92	0.85 ±0.03	0.14	0.12	0.13	0.12 ±0.04

±: Standard error

Table:3. Estimation of carbohydrate and protein contents before adding and after separating the organisms from whey

Organism	Liquid whey		Powder whey	
	%age carbohydrate decreased	%age protein decreased	%age carbohydrate decreased	%age protein decreased
Bacteria				
Bacillus subtilis	50.0 ±3.54	62.0 ±2.05	54.0 ±2.57	74.0 ±1.50
Bacillus sp1	30.0 ±5.76	75.0 ±0.51	27.0 ±3.01	78.0 ±1.85
Bacillus sp2	60.0 ±2.97	73.0 ±0.49	47.0 ±2.92	67.0 ±2.92
Yeast				
Candida sp	57.1 ±3.52	53.0 ±0.52	60.0 ±2.01	56.0 ±2.01
Saccharomyces sp	71.0 ±5.12	47.0 ±1.29	54.0 ±3.95	63.0 ±2.47
Fungi				
Aspergillus flavus	33.0 ±4.95	42.0 ±1.05	27.0 ±3.09	48.0 ±1.05
Aspergillus niger	50.0 ±3.25	37.0 ±1.45	20.0 ±2.87	50.0 ±0.84

*LC1, PC1: carbohydrate contents before adding the organism in whey

**LC2, PC2: carbohydrate contents after separating the organism from whey

±: Standard error

Determination of Carbohydrate in Liquid and Powder Whey: The carbohydrate contents were measured by phenol sulphuric acid method and taking the (OD) optical density at 490 nm. The initial carbohydrate contents in liquid whey before adding organism were found to be 10 OD ,14 OD and 12 OD (Table 3), however after adding the organisms in the liquid whey medium and passing specific period of time required for the growth of organisms, the carbohydrate contents were significantly utilized by fungi and yeast i.e. up to 70% (Table 3) while in case of bacteria up to maximum 60% carbohydrate contents were used while minimum consumption of carbohydrates was recorded to be 30% in case of *Bacillus* sp 1. In case of powder whey dissolved in distill water, the maximum utilization of carbohydrate contents were observed in *Candida* and *Saccharomyces* species which were 60% and 54% respectively, while least consumption was observed in fungi; *Aspergillus flavus*, *Aspergillus niger* i.e., 27% and 20% respectively (Table 3). The present study indicates that yeast is better consumer for carbohydrates than bacteria and fungi.

Whey is a by product of the diary industry and contains usually high levels of lactose and low levels of nitrogenous compounds as well as vitamins and nutrients (Giec and Skupin, 1988). The recent study the utilization of whey media was studied for the production of biomass of different organism's viz., bacteria, yeast and fungi. The bacteria were found to be most efficient in protein utilization from whey media than both yeast and fungi because their cell division time is faster as compared to yeast and fungi which are considered to be slow growing organisms (Table 3). The results reported here in present study were similar pattern as described by Kim et al. (1981).

The yeast strains were found to be more efficient regarding the consumption of carbohydrate contents as compared to bacteria as well as fungi (Mawson, 2004). The fungal strains indicated least efficiency regarding the utilization of carbohydrate contents perhaps due to reason that fungi amino acid profile is better and they use it as primary energy source than carbohydrates The maximum SCP biomass was observed in liquid whey in case of fungal strains while least was found in liquid whey in bacterial strains. While on the other hand, in powder whey highest SCP biomass was recorded in yeast and fungi because these organisms were although relatively slow growing but they showed higher SCP biomass production due to relatively larger size of the cell and efficient consumption of carbohydrate source especially in case of yeast. These results were supported by Omar and Sabry (1991).

CONCLUSION: The recent study described that fungi and yeasts were the most efficient as regarding SCP biomass production as compared to bacteria. However regarding the utilization of protein contents of whey bacteria are more efficient than yeast and fungi. In case of carbohydrates utilization from whey, yeast and bacteria are more effective micro-organism than fungi.

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