RESEARCH ARTICLE

Liquid Whey: A Potential Substrate for Single Cell Protein Production from *Bacillus subtilis* NCIM 2010

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ABSTRACT

The term single cell protein (SCP) refers to dead, dry cells of micro-organisms. Single Cell Protein production was carried out from liquid whey by using *Bacillus subtilis.* It was observed that all the whey samples produced the Single Cell Protein (SCP). In the present study, out of the 5 different whey samples, the whey sample-4 has shown high Single Cell Protein production (0.32 mg/ml) followed by whey sample-3 (0.28mg/ml), whey sample-2 (0.24mg/ml) and whey sample-1 and 5 has shown (0.16 mg/ml each) of SCP production. Thus liquid whey can be used for single cell protein production.

Keywords: Single Cell Protein**,** *Bacillus subtilis*, Liquid Whey

INTRODUCTION

Microorganisms play important role in the production of high protein food content such as cheese and fermented soyabean products from ancient time (Adedayo *et al*., 2011). , Protein being the main nutritional component in both types. Since a large proportion of cell dry weight is accounted for protein, the nutritional value of a microbial derived food source is determined by the levels of protein produced (Patel, 1995). The development of microbial systems for use in food industry has firstly; growth of microorganism is very much faster than of animals. Secondly, a broader range of materials may be considered as suitable substrates depending on the microorganism chosen. The two chief strategies with regard to substrate to consider low grade waste material or to use relatively simple carbohydrate source to produce microbial material containing very high quality of protein (Reed and Nagodawithana, 1995).

Microorganisms can utilize waste materials that cause pollution problem and also sanitary hazards. The use of wastes would help in controlling pollution and also in solving waste disposal problem to some extent. Single Cell Protein (SCP) production has the potential for feeding the ever increasing world population at cheaper rates (Baldasso *et al*., 2011). The protein comes from a number of vegetables, cereals and fruits, often not affordable by a common man and therefore microbial proteins can be an alternative source to feed economically down communities in the world in general and India in particular. In other words proteins are the essence of life processes and are important for proper growth and development of all the living beings. Its deficiency may lead to a number of health disorders. The term single cell protein refers to dead, dry cells of micro-organisms such as yeast, bacteria, fungi and algae which grow on different carbon sources (Anupama and Ravindra, 2000). Single cell protein typically refers to source of mixed protein extracted from pure or mixed culture of algae, yeast, fungi or bacteria (Khan *et al*., 2010; Ware, 1977). In present investigation, a simple process for utilizing whey protein and converting lactose to SCP in order to obtain product which enhance the biological value of microorganism *Bacillus subtilis*. This bacteria is screened for its growth and SCP production using whey as a substrate.

Whey has the importance in dairy industry due to its nutritional value. It is produced in large amount as the by-product of cheese or casein production. A total of 180 to 190x10^6 ton/year whey production was carried out worldwide, in which only 50% is processed (Panesar *et al*., 2007). From this, half of the amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its byproducts and remaining as cheese whey-protein concentrates (Spalatelu, 2012). However it is not utilized for further lactose production; so that whey disposal represents a serious problem from economical as well as an environmental point of view. On the other hand whey is beneficial for the environment and also for a suitable economy (Jelen, 2003).

Whey is likely the most popular protein supplement in the market. Whey has lots of applications in the food and pharmaceutical industries. It is the need to formulate novel and alternative food to supply. The production of Single Cell Protein is a major step in this direction. The utilization of whey as protein substrate from bacteria was found to be more efficient. The aim of the present study was to evaluate the liquid whey as a potential substrate for Single Cell Protein production from *Bacillus subtilis* NCIM 2010.

MATERIALS AND METHODS

Inoculum Preparation:

Liquid whey samples were obtained from five different market places of Nagpur city. The samples were transferred to 1 litre sterilized plastic bottle. These bottles were stored in refrigerator until required. *Bacillus subtilis* NCIM 2010 was inoculated on Luria Bertani media plates and incubated at 37^0C. After 48 hours of incubation the culture was inoculated in 250 ml conical flask having 50 ml whey sample each. The flasks were kept at 37^0C on Rotary Shaker Incubator for 2 days. The whey samples were centrifuged at 6000 rpm for 10 minutes. The supernatant obtained from all the whey samples were further used as an unknown sample for estimation of single cell protein by using Lowry's method (Lowry *et al*., 1951).

Estimation of Protein by Lowry's Method:

All the tubes were kept at room temperature for 10 minutes, and then 0.5ml of 1:2 dilution of Follin's reagent was added rapidly and mixed thoroughly. After keeping for 30 minutes at room temperature the optical density was observed at 660 nm. Protein was estimated using following formula. The amount of protein present in the given whey samples was estimated from the standard graph. Concentration of Protein = Optical Density (OD)/ Bovine Serum Albumin (BSA) addition (ml).

Table 1: The protocol for protein estimation is given below.

Sr. No.	Reagents	B (ml)	T1 (ml)	T2 (ml)	T3 (ml)	T4 (ml)	T5 (ml)	T6 (ml)	T7 (ml)	T8 (ml)	U (ml)
1.	BSA	-	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	-
2.	Whey sample	-	-	-	-	-	-	-	-	-	0.8
3.	Distilled water	2	1.8	1.6	1.4	1.2	1	0.8	0.6	0.4	0.2
4.	Alkaline $CaSO_4$	5	5	5	5	5	5	5	5	5	5

RESULTS AND DISCUSSION

The protein amount in whey sample was determined by Lowry's method for Single Cell Protein production from the different whey samples. In the present study 5 different whey samples were collected from five different market places in Nagpur city. The samples were further proceeded for SCP production by using *Bacillus subtilis* NCIM 2010. It was observed that all the whey samples produced the Single Cell Protein. In the present study, out of the 5 different whey samples, the whey sample-4 has shown high Single Cell Protein production (0.32 mg/ml) followed by whey sample-3 (0.28mg/ml), whey sample-2 (0.24mg/ml) and whey sample-1 and 5 has shown (0.16 mg/ml each) of SCP production (Table 2, 3) (Figure 1-5). The obtained results were correlated with Ahmad *et al*., (2009). Whey is a byproduct of the dairy industry and contains whey proteins, lactose, water-soluble vitamins and minerals.

Whey has lots of applications in the food and pharmaceutical industries (Baldasso *et al*., 2011; Koller *et al*., 2005). Whey proteins having high nutritional value, due to the involvement of sulfur-containing amino acids (De Wit, 1998). Siso (1996) reported that whey has the protein efficiency ratio (PER) of 3.4 which is higher than casein (2.8) and similar to egg albumin. Moreover, Gunasekaran *et al*., (2007) demonstrated that whey proteins possess important functional properties such as high solubility, water absorption, gelatinization and emulsifying capacities which are essential in food application and is the cheaper source.

In the present study *Bacillus subtilis* was taken for SCP production by inoculating it in the whey samples and was found to be promising in the SCP production. The study of Kim *et al*., (1981) also revealed the similar findings by using *Bacillus subtilis*. *Bacillus subtilis* was found to be most efficient in protein utilization from whey than both yeast and fungi because its cell division time is faster as compared to yeast and fungi which are considered to be the slow growing organisms. However, Huei-hsiung (1976) used the bacteria *Brevibacterium* for the production of SCP.

Table 2: Estimation of Single Cell Protein (SCP) from Whey Samples

Whey Sample	O.D. (nm)	Concentration (mg/ml)
Whey 1	0.20	0.16 mg/ml
Whey 2	0.30	0.24 mg/ml
Whey 3	0.35	0.28 mg/ml
Whey 4	0.40	0.32 mg/ml
Whey 5	0.20	0.16 mg/ml

Table 3: Estimation of Single Cell Protein (SCP) from Whey Samples by Using *Bacillus subtilis*

Test Tube	Whey 1		Whey 2		Whey 3		Whey 4		Whey 5	
	OD(nm)	Conc.	OD(nm)	Conc.	OD(nm)	Conc.	OD(nm)	Conc.	OD(nm)	Conc.
B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T1	0.9	0.04	0.20	0.04	0.22	0.04	0.09	0.80	0.09	0.04
T2	0.9	0.08	0.10	0.08	0.09	0.08	0.10	0.08	0.15	0.06
T3	0.12	0.12	0.25	0.20	0.25	0.20	0.15	0.09	0.25	0.20
T4	0.21	0.16	0.20	0.16	0.32	0.25	0.25	0.20	0.32	0.25
T5	0.24	0.20	0.35	0.35	0.23	0.23	0.12	0.12	0.24	0.24
T6	0.29	0.24	0.25	0.30	0.32	0.38	0.20	0.24	0.32	0.38
T7	0.32	0.28	0.30	0.42	0.35	0.49	0.25	0.35	0.32	0.28
T8	0.42	0.32	0.30	0.48	0.20	0.32	0.28	0.44	0.41	0.32
U	0.20	0.16	0.30	0.24	0.35	0.28	0.40	0.32	0.20	0.16

Where, B= Blank, T= Test tube, U= Unknown, OD= Optical Density, Conc.= Concentration

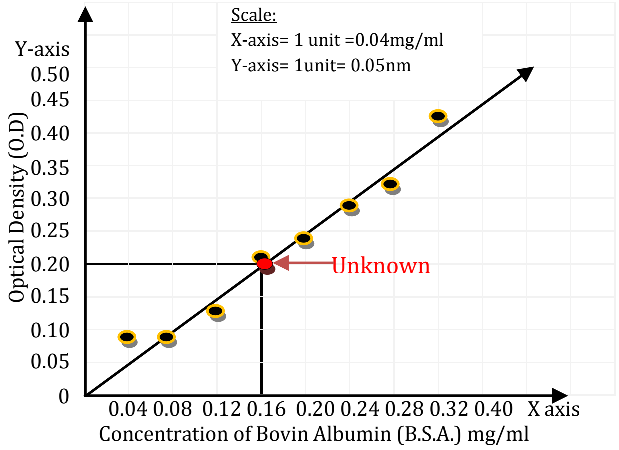

Fig. 1: Estimation of SCP from whey Sample -1 by using *Bacillus subtilis*

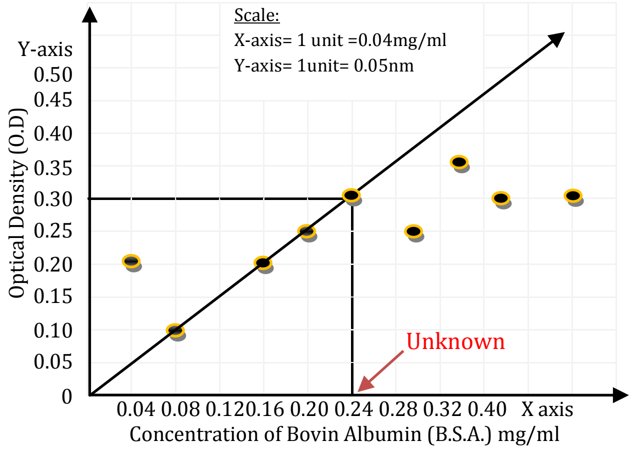

Fig. 2: Estimation of SCP from whey Sample -2 by using *Bacillus subtilis*

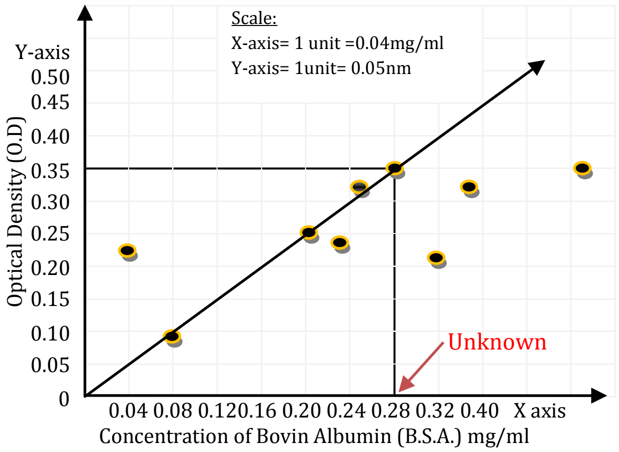

Fig. 3: Estimation of SCP from whey Sample -3 by using *Bacillus subtilis*

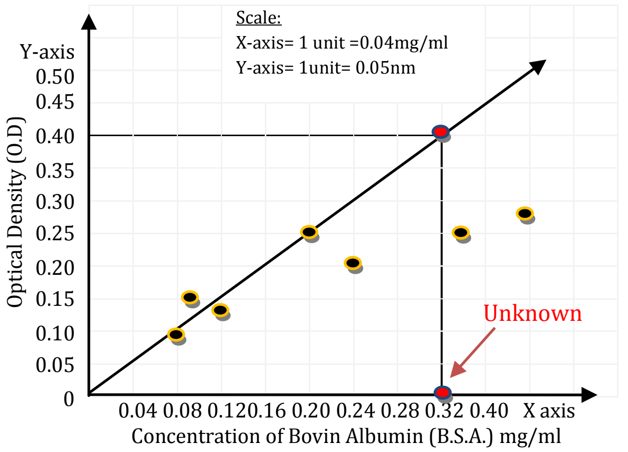

Fig. 4: Estimation of SCP from whey Sample -4 by using *Bacillus subtilis*

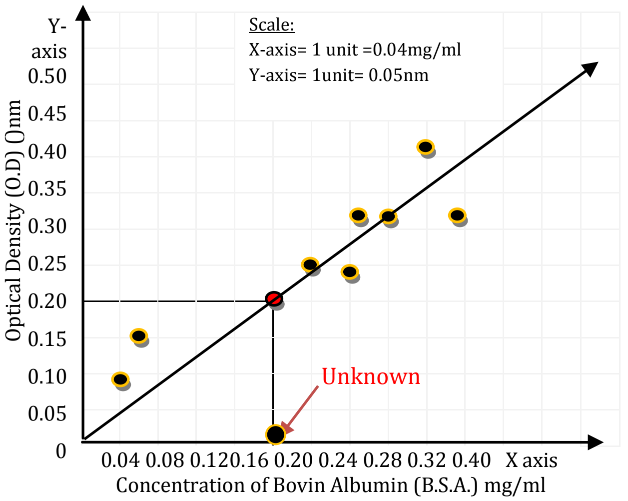

Fig. 5: Estimation of SCP from whey Sample -5 by using *Bacillus subtilis*

In the present study liquid whey samples were taken for SCP production. According to Omar and Sabry (1991), the maximum SCP biomass was observed in liquid whey in bacterial strains is 62%. Several different fruit wastes have uitilized as substrate by a number of researchers such as Kamel (1979) used dates, Ghanem (1992) used beet pulp, Azin and Moazami (1989) used sugarcane bagasse, Enwefa (1991) used banana skins and Moharib (2003) used guava peel for the production of single cell protein. Thus in their investigation different combinations of fruit wastes were explored for biomass production (SCP) instead of dumping them. According to Marshall (2004) now day's whey is the popular dietary protein which supports for antimicrobial activity, immune modulation and for the prevention of cardiovascular disease and osteoporosis. Whey also serves as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial and chelating agent.

CONCLUSION

The present study concluded that Single Cell Protein (SCP) production was carried out efficiently from liquid whey samples by using *Bacillus subtilis*. In the present study, out of the 5 different whey samples, the whey sample-4 has shown high Single Cell Protein production (0.32 mg/ml) followed by whey sample-3 (0.28mg/ml), whey sample-2 (0.24mg/ml) and whey sample - 1 and 5 has shown (0.16 mg/ml each) of SCP production. The use of Single Cell Protein (SCP) as food ingredient is still in its stages of development, there are lots of prospects concerning the improvement of using SCP as food, methods of using genetic engineering procedure for mass production of these protein containing microorganisms are been employed. Attempt to improve the acceptability of SCP products should be intensified. Further research and development will ensure usage of microbial biomass as Single Cell Protein or as diet in supplement.

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