International Journal of Current Research in Multidisciplinary (IJCRM) *ISSN: 2456-0979*
Vol. 5, No. 1, (January'20), pp. 28-34

PRODUCTION OF LIQUID BIOFERTLIZERS FROM COTTON CHEESE WHEY

Ms. Moghana A & Dr. V. Judia Harriet Sumathy
PG & Research Department of Biotechnology, Women's Christian College, Chennai – 600 006

ABSTRACT

In olden days, whey was used for treatment of health related reasons. Hippocrates (446 - 337BC), the Father of Medicine prescribed whey to his patients. Later Galen (131BC – 200BC), also advised whey to his patients. A patient who could not be cured by doctors was sent to the Swiss mountain village of Gais where he got healed by drinking the whey every day. As the news spread people came to Gais to benefit from the properties of whey protein. A health spa was opened in that small village followed by more than 160 spas across Switzerland, Austria and Germany. These spa were active throughout the eighteenth and nineteenth century. Biofertilizers are substances which contains living microorganisms which when applied to seeds, soil, plant surfaces promotes growth by increasing the supply of the availability of the micro minerals. Bio fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Biofertilizers can be expected to reduce the use of synthetic fertilizers and pesticides. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts and do not contain any chemicals which are harmful to the living soil. The present study aims at producing Liquid Biofertilizers from Cotton Cheese Whey.

KEYWORDS: *Cotton Cheese Whey, Patient, Health Spa, Treatment and Liquid Biofertilizers.*

INTRODUCTION

Whey is the liquid that is obtained after the milk is curdled. After straining the curdled milk the whey alone is separated and is considered as the waste. The whey collected after the production of cheese is known as cheese whey (**Martin Koller *et.al.*, 2012**). The whey is of two types namely, sweet whey and acid whey. The sweet whey is produced during the production of hard cheese like Swiss cheese and the acid whey is produced during the production of Cottage cheese (**Luisina Lavari *et.al.*, 2014**). It can be considered as 5% solution of lactose in water, with few minerals and lactalbumin (**Adeela Yasmin *et.al.*, 2013**). Whey can be denatured by high heat as above 72˚C. It has primary ingredients of proteins and thus athletes and body builders are supplemented with it in their diet **(Vethakanraj Helen Shiphrah *et.al.*, 2013**). Whey protein has high amount of leucine thus helping muscle growth and repair (**Gonzfilez Siso MI.1996**).

TYPES OF WHEY PROTEIN

Whey protein is available in four main forms.

- **Concentrate** : It has low fat and cholesterol but high carbohydrates in the form of lactose protein in weight 29% -89%.
- **Isolate** : when the fat and lactose are removed, 90% of it accounts for protein by weight.
- **Hydrolysate**: They are predigested and partially hydrolyzed to be easily metabolized. But the cost is higher. It is less allergic than other forms.
- **Native**: Extracted from skim milk, not from cheese (**Nivedita Sharma and Neha Gautam. 2007, Hermann L. 2013, Haron SA and Ibrahim AH. 2003 and Itelima *et.al.*, 2018**) .

WHEY CREAM AND BUTTER

From whey, the cream can be skimmed which is saltier, tangier and cheesier. Because of low fat content, the yield is low. They are cheap to manufacture as sweet cream and butter. Thus in olden days the whey cream and butter was mostly used for its nutritional purpose (**Nikolay Vassilev *et.al.*, 2015, Kecskes *et.al.*, 2015, Maria Julia Estrella *et.al.*, 2014 and Emilce Viruel *et.al.*, 2009**).

ENVIRONMENTAL IMPACT

Untreated whey when disposed into the lakes and rivers pollutes the water bodies. The water becomes unfit for drinking as well as for the survival of biotic species. The cause for such pollution is due to high level of Biological Oxygen Demand and Chemical Oxygen Demand in the disposed whey (**Willibrordus Augustinus Van der Weide. 2003**). The continued practice of untreated whey into the environment could cause severe damage to the environment as well as the living organisms. Thus one of the best way to resolve the problem is to convert the untreated whey into a biofertilizer. It helps as a resource for the welfare of the environment by preventing the harmful effect of the chemical fertilizers on plants as well as the soil (**Peter crisp *et.al.*, 2014**).

MATERIALS AND METHODOLOGY

SAMPLE COLLECTION

The cottage cheese whey was collected from Small Dairy Industry at Maraimal Nagar. BOD and COD level were estimated. The *Lactobacillus* isolated from Curd was subjected to Gram Staining and was subcultured in MRS media. The sample was fermented for a month and was centrifuged and the BOD, COD, and Antibacterial Activity were estimated.

RESULTS AND DISCUSSION

BIOLOGICAL OXYGEN DEMAND VALUE OF THE COTTAGE CHEESE WHEY

BOD is expressed as weight of oxygen consumed per unit volume of the sample**.** It is related to the amount of biodegradable organic matter present in the sample. The method consists of placing a sample in a BOD bottle. Dissolved oxygen is measured initially and after incubation. The BOD is calculated from the difference between the initial and final dissolved oxygen. Moderately polluted rivers may have a BOD value in the range of 2 to 8 mg/L (**Figure 1**).

Calculation

$$\text{BOD value} = \frac{T*N*1000*Eq.}{V} = \frac{8.5*0.1*1000*8}{50} = 136 \text{ mg/L}$$

where, T: volume of sodium thiosulphate
N: normality of sodium thiosulphate
V: volume of sample taken
Eq.: Equivalent weight of oxygen

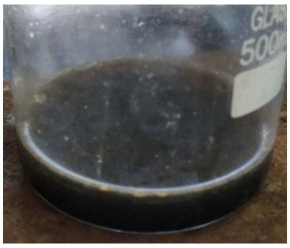

Figure 1 : BOD of Cotton Cheese Whey

CHEMICAL OXYGEN DEMAND VALUE OF THE COTTAGE CHEESE WHEY

A known volume of potassium dichromate oxidizes the organic matter and then excess oxygen is allowed to react with potassium iodide to liberate iodine in amounts equal to the excess oxygen which is estimated titrimetrically with sodium thiosulphate solution using starch as indicator (**Figure 2A & B**).

Calculation

$$\text{COD value} = \frac{T*N*1000*Eq.}{V} = \frac{63*0.1*1000*8}{50} = 1008 \text{ mg/L}$$

where, T: volume of sodium thiosulphate
N: normality of sodium thiosulphate
V: volume of sample taken
Eq.: Equivalent weight of oxygen

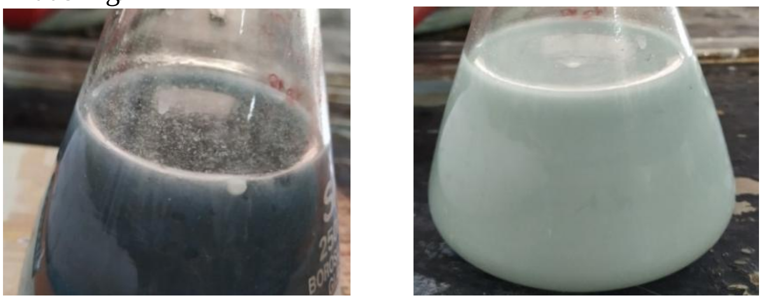

Figure 2 A & B : COD of Cotton Cheese Whey

ISOLATION AND IDENTIFICATION OF *Lactobacillus* **FROM CURD**

Lactobacillus is a kind of bacteria which can convert a sugar into an alcohol and then into an acid by means of anaerobic respiration. MRS agar (deMan, Rogosa and Sharpe) supports luxuriant growth of all *lactobacillus*. It has proteose peptone and beef extract supply which is nitrogenous and carbonaceous. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of *lactobacillus*. They appear as white colonies (**Figure 3A & B**).

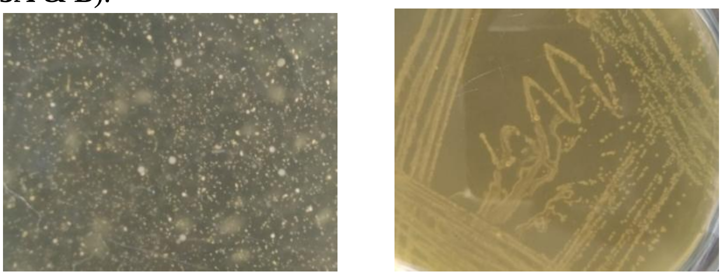

Figure 3 A & B : Isolation and Identification of *Lactobacillus* f**rom Curd**

GRAM STAINING OF THE ORGANISM

Lactobacillus is a genus of gram- positive, facultative anaerobic or microaerophilic, rod- shaped, non-spore forming bacteria. The cell walls of gram positive bacteria have a thick layer of protein- sugar complexes and thus the crystal violet stain is not decolourized (**Figure 4**).

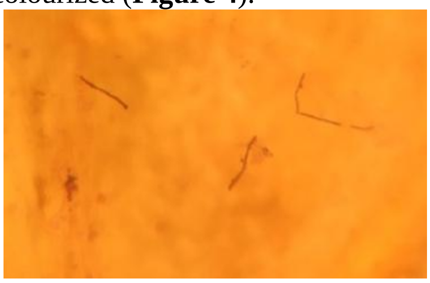

Figure 4 : Gram Staining

LIQUID BIOFERTILIZER FROM FERMENTED SAMPLE

The supernatant that is obtained after the cold centrifugation can be used as the liquid biofertilizer. It can be preferred rather than using chemical biofertilizers. Since it is from a natural source, it will benefit the environment. The liquid biofertilizer will give good yield in the production of plants. The collected liquid biofertilizer can be stored better in air tight container (**Figure 5**).

Figure 5 : Liquid Biofertilizer from Fermented Sample

SOLID BIOFERTILIZER FROM FERMENTED SAMPLE

The pellet that is obtained after the cold centrifugation can be used as the solid biofertilizer. The pellet is dried and powdered. The amount of pellet obtained is very less in amount. For 250ml of the sample kept for fermentation only 0.5g of the powdered solid biofertilizer was obtained. It can serve as a very good source of nutrient for the plant growth (**Figure 6**).

Figure 6 : Solid Biofertilizer from fermented Sample

BIOLOGICAL OXYGEN DEMAND VALUE OF THE FERMENTED WHEY

BOD is expressed as weight of oxygen consumed per unit volume of the sample**.** It is related to the amount of biodegradable organic matter present in the sample. The method consists of placing a sample in a BOD bottle. Dissolved oxygen is measured initially and after incubation. The BOD is calculated from the difference between the initial and final dissolved oxygen. Moderately polluted rivers may have a BOD value in the range of 2 to 8 mg/L (**Figure 7**).

Calculation

$$\text{BOD value} = \frac{T*N*1000*Eq.}{V} = \frac{14*0.1*1000*8}{50} = 224\text{mg/L}$$

where, T: volume of sodium thiosulphate
N: normality of sodium thiosulphate
V: volume of fermented sample taken
Eq.: Equivalent weight of oxygen

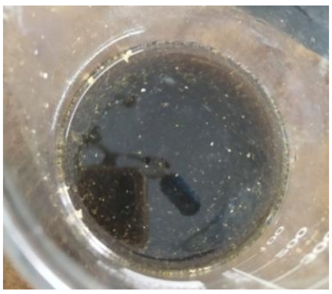

Figure 7 : BOD of Fermented Whey

CHEMICAL OXYGEN DEMAND VALUE OF THE FERMENTED WHEY

A known volume of potassium dichromate oxidizes the organic matter and then excess oxygen is allowed to react with potassium iodide to liberate iodine in amounts equal to the excess oxygen which is estimated titrimetrically with sodium thiosulphate solution using starch as indicator (**Figures 8A – C**)..

Calculation

$$\text{COD value} = \frac{T*N*1000*Eq.}{V} = \frac{18.75*0.1*1000*8}{50} = 300 \text{ mg/L}$$

where, T: volume of sodium thiosulphate
N: normality of sodium thiosulphate
V: volume of fermented sample taken
Eq.: Equivalent weight of oxygen

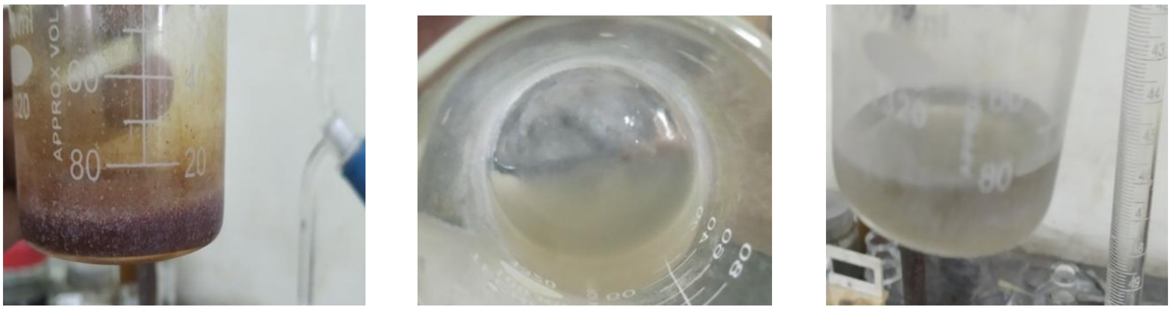

Figure 8 A – C : COD of Fermented Whey

ANTIBACTERIAL ACTIVITY

The antibacterial activity was checked for the fermented whey against five microorganisms such as *Staphylococcus Spp*, *Streptococcus Spp*, *Pseudomonas Spp*, *Klebsiella Spp*, and *Escherichia coli.* Absence of Antibacterial activity was reported. No zone of inhibition was observed after 48 hours of incubation at 37˚C (**Figure 9**).

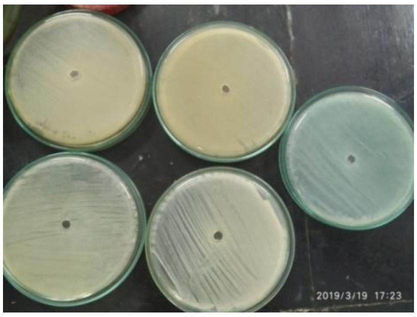

Figure 9 : Antibacterial Activity

QUALITY TESTING OF THE LIQUID BIOFERTILIZER

The liquid whey sample was given for testing in order to check the presence of few micro-minerals and macro-minerals as well as to quantify the amount of minerals present. The Results indicate the presence of Total Nitrogen, Total Phosphate, Potassium, Zinc, Manganese, Iron and Copper. Hence the product can serve as a good Liquid Biofertilizer.

S. No.	Parameters	Units	Results on As-Is Basis	Test Method
1	pH	-	3.30	Biofertilizers and Organic Fertilizers in the Fertilizer (Control) Order 1985, Schedule IV - Part D. Methods of Analysis of Soils, Plants, Waters, Fertilizers and Organic Manures ; edited by HLS Tandon (FDCO)
2	Electrical Conductivity	µS/cm	8520	
3	Total Nitrogen (Kjeldahl)	%	0.03	
4	Total Phosphate as P_2O_5	%	0.21	
5	Potassium as K_2O	%	0.15	
6	Zinc as Zn	mg/Kg	2.69	
7	Manganese as Mn	mg/Kg	<DL (0.1)	
8	Iron as Fe	mg/Kg	9.73	
9	Copper as Cu	mg/Kg	1.24	

Parameters	Units	Results on As-Is-Basis	Test Method
pH	-	**3.30**	Biofertilizers and Organic Fertilizers in The Fertilizer (Control) Order 1985, Schedule IV - Part D Methods of Analysis of Soils, Plants, Waters, Fertilisers & Organic Manures; Edited by HLS Tandon (FDCO)
Electrical Conductivity	µS/cm	**8520**	
Total Nitrogen (Kjeldahl)	%	**0.03**	
Total Phosphate as P_2O_5	%	**0.21**	
Potassium as K_2O	%	**0.15**	
Zinc as Zn	mg/Kg	**2.69**	
Manganese as Mn	mg/Kg	**<DL(0.1)**	
Iron as Fe	mg/Kg	**9.73**	
Copper as Cu	mg/Kg	**1.24**	

Authorised Signatory

R Kalpana

Quality Manager

X----------X

CONCLUSION

The cottage cheese whey was collected from a Small Dairy Industry from Maraimal nagar to process it and convert it into a valuable resource. The collected cottage cheese whey was subjected to test for its Biological Oxygen Demand and the value was found to be high than the normal range. The collected cottage cheese whey was subjected to test for its Chemical Oxygen Demand and the value was found to be higher than the normal range. Compared to BOD, the COD value was found to be higher. The organism *Lactobacillus Spp* was isolated from two dilutions of the curd in distilled water. The dilutions 10^{-4} and 10^{-5} showed better results. Creamy white colonies were obtained on MRS (deMan, Rogosa and Sharpe) media. The obtained culture was gram stained and observed under microscope under 10x, 40x, and 100x oil immersion. Gram positive, long rods were observed. After confirmation, the culture was streaked on MRS media with the help of a loop. The petriplate was incubated and the colonies were observed after 48 hours and were kept at 37ºC. The obtained culture was developed in the MRS broth and placed inside the shaker for 24 hours. This broth was used as starter culture. The cottage cheese whey was taken in conical flask and placed inside the shaker for a month after the addition of the starter culture into the whey. Biomass production was observed. The fermented cottage cheese whey was taken in centrifuge tubes and centrifuged to collect the supernatant and the pellet. The supernatant was used as Liquid Biofertilizer and the pellet as Solid Biofertilizer. The fermented cottage cheese whey was subjected to test for its Biological Oxygen Demand and the value was found to be high than the normal range due to microbial growth. The fermented cottage cheese whey was subjected to test for its Chemical Oxygen Demand and the value was found to be decreased when compared to the COD value of unfermented cottage cheese whey. Anti - bacterial test was performed using the fermented cottage cheese whey and no zone of inhibition was observed. Thus this study presents a strategy as to how industrial waste streams like large amount of whey can be upgraded to the role of substrates of producing valuable products such as biofertilizers. This would contribute a great deal to the agricultural industry in order to eliminate the toxicity that is usually produced by chemical fertilizers. Improvements in the fermentation strategy by switching from discontinuous to continuous mode can yield better quality biofertilizers. Thus the present study can be applied for large scale production by Industries to benefit from the whey which is an Environment threat and otherwise wasted.

BIBLIOGRAPHY

[1] *Martin Koller, Anna Salerno, Alexander Muhr, Angelika Reiterer, Emo Chiellini, Sergio Casella, Predrag Horvat, Gerhart Braunegg.2012. Whey Lactose as a Raw Material for Microbial Production of Biodegradable Polyesters, 2 : 36 - 40.*

[2] *Luisina Lavari, Alejandra Cuatrin, Roxana Paez, Jorge Reinheimer and Gabriel Vinderola. 2014. Use of cheese whey for biomass production and spray drying of probiotic lactobacilli, 2 : 2 - 3.*

[3] *Adeela Yasmin , Masood Sadiq Butt , AyshaSameen, Muhammad Shahid. 2013. Physicochemical and Amino Acid Profiling of Cheese Whey, 3 : 456 – 457.*

[4] *Vethakanraj Helen Shiphrah, SayantiSahu, AshokeRanjan Thakur, Shaon Ray Chaudhuri 2013. Screening of Bacteria for Lactic Acid Production from Whey Water, 2 : 118 -120.*

[5] *Gonzfilez Siso MI.1996. The Biotechnological Utilization of Cheese Whey: A Review, 2 : 1- 8.*

[6] *Nivedita Sharma, Neha Gautam. 2007. Antibacterial activity and characterization of bacteriocin of Bacillus mycoides isolated from whey, 4 : 117 - 121.*

[7] *Hermann L. 2013. Challenges of formulation and quality of biofertilizers for successful inoculation, 2 : 1 - 2.*

[8] *Haron SA, Ibrahim AH. 2003. Whey Induced- Modifications in Growth, Photosynthetic Characteristics, Protein patterns and Water Relations of Wheat Seedlings, 2 : 141 – 153.*

[9] *Itelima JU, Bang WJ, Sila, Onyimba IA, Egbere OJ. 2018. A review: Biofertilizer – A enhancing soil fertility and crop productivity, 1 : 1 – 5.*

[10] *Nikolay Vassilev, Maria Vassileva, Lopez A, Vanessa Martos. 2015. Unexploited potential of some biotechnological techniques for biofertilizer production and formulation, 2 : 1 - 2.*

[11] *Kecskes ML, Choudhury ATMA, Casteriano AV, Deaker R, Roughley RJ, Lewin L. 2015. Effects of bacterial inoculants biofertilizers on growth, yield and nutrition of rice in Australia, 2 : 377 - 380.*

[12] *Maria Julia Estrella, Fernando Luis Pieckenstain, Maria Marina, Diaz LE. 2004. Cheese whey: An alternative growth and protective medium for Rhizobium loti cells, 3 : 1- 2.*

[13] *Emilce Viruel, Faustino sineriz, Maria E Lucca. 2009. Biofertilizer production bioprocess using vinase and cheese whey in the media composition, Volume 2, pages 11-12.*

[14] *Willibrordus Augustinus Van der Weide. 2003. Method for the production of biological fertilizer containing whey, 2 : 2 - 3.*

[15] *Peter crisp, Wicks TJ, Michelle Lorimer, Eileen S Scott. 2014. An evaluation of biological and abiotic controls of grapevine powdery mildew, 2 : 3 - 5.*

