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PRODUCTION OF YEAST ON CHEESE WHEY

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ABSTRACT

Whey generated in cheese manufacturing processes serious environmental issues that limit process profitability. The dairy industry's innovation recognises the "bio-refinery" as a key to successful whey disposal and economic growth. The valorisation of cheese whey is a multi-technological process that can result in value-added products (biomass, fine or bulk chemicals).The aim of this research is to improve a fermentation method that uses whey as a growth medium and carbon source.Lactose, which is abundant in whey, is an important carbon source. However, the yeast *Saccharomyces cerevisiae*, which is more commonly utilised in commercial fermentation processes, is not a lactose-fermenting yeast.We designed a novel biotechnological technique for the large-scale synthesis of non-genetically modified yeast biomass that may be employed in a variety of applications, such as bread making, production of probiotics, nutraceuticals, bio-active molecules. To use cheese whey as a raw material for *S. cerevisiae* productionand to overcome the limitations in the use of lactose we used externally added substrates such as peptone, yeast extract and casein. The careful optimization of the amount of additional substrate allowed for the slow release of glucose and galactose via hydrolysis and simultaneous consumption, resulting in a decrease in ethanol and an increase in biomass produced.

Keywords: *Saccharomyces cerevisiae*, Cheese whey, Fermentation, Yeast.

INTRODUCTION

The global production of cheese whey is estimated to be around 108 t/year. It can't just be utilised as animal feed; it might be an appealing raw material for the manufacturing of a wide range of value-added compounds (Mollea,2013), making whey not just a waste but also a profitable resource.Whey is a turbid greenish-yellow liquid that makes up the watery part after fat and caseins have been separated from whole milk. Primary constituents of whey are mainly water (92 %), lactose (5 %), whey proteins and fats (1.9 %) and soluble salts (0.9 %).Chemical, physical, and microbiological characteristics vary greatly depending on the manufacturing procedure (fresh or hard cheeses), the milk source (cow or goat), and the season of production (González,1996).*Saccharomyces cerevisiae* is one of the oldest industrial fermentation products, and it remains one of the most important biotechnological products due to its wide range of applications. Simple sugars, particularly sucrose, as well as sugar beet molasses or sugar cane, are currently employed as substrates for producing yeast biomass.Cheese whey can be utilized as a growth medium and carbon source due to its complex chemical

makeup. It is also affordable and readily available (pisano,2015).
Indigenous milk enzymes, the coagulant, and starter and nonstarter microorganisms are all sources of proteolytic enzymes in cheese.During maturation, chymosin is responsible for the early hydrolysis of casein (Fox et al., 1996). Plasmin, a naturally occurring milk enzyme, is also involved. Large and intermediate-sized peptides are produced by these enzymes. Lactococcus lactis, the starter culture, contains a cell envelope-associated proteinase (CEP) and a battery of intracellular peptidases.After importation into the cell or cell lysis, the CEP hydrolyzes intermediate molecular weight peptides into low molecular weight peptides, which are then degraded to free amino acids by intracellular peptidases (Kunji et al., 1996; Mierau et al., 1996; Christensen et al., 1999).Peptidolysis does not readily occur in cheese until the starter bacteria lyse, releasing intracellular peptidases into the cheese matrix, where they have direct access to their peptide substrates (Boutrou et al., 1998).These peptidases hydrolyze low-molecular-weight peptides into free amino acids, which can then be used in other chemical reactions.

Cheese whey disposal necessitates a substantial amount of industrial capital to avoid major environmental problems due to its high biological (40,000 ppm) and chemical oxygen demand (60,000 ppm), primarily due to high lactose content (Ghaly,2007). As a result, finding a solution to the whey disposal problem is evident, and the "biorefinery," in which residual biomass, such as whey, is used to produce a variety of products such as animal feed, bulk and fine chemicals, could be the key to success.The future trend for cheese factories is to move towards zero discharge, which means avoiding expensive disposal costs and finding more environmentally friendly and economic uses for lactose. Sansonetti (2010) described a number of techniques in which yeasts or active microbial biomasses like lactic acid starters and baker's yeast are employed to make bioethanol.

MATERIALS AND METHODS

1. Sample Preparation: Cheese whey was obtained by adding 2ml of lemon juice in 500ml milk. Whey was separated from milk using muslin cloth. Use this whey as a raw material for production of yeast.

2. Enrichment of Culture: Take 5 conical flasks and label them as A,B,C,D,E.Fill flasks A and B with 50 mL of whey.Weigh 1g of peptone powder dissolved it in small amount of whey and make up the volume 50ml (flask C).Weigh 1g of yeast extract powder dissolved it in small amount of whey and make up the volume 50ml (flask D).Weigh 1g of casein powder dissolved it in small amount of whey and make up the volume 50ml (flask E).Autoclave all 5 flasks at 15lbs pressure for 15 mins.Add 1g of Baker's yeast in flask B, C, D and E. Incubate the flasks at room temperature and observe them after every 24 hrs.

3. Wet weight: Take 5 centrifuge tubes and labelled them as A,B,C,D,E note their weight.Add 5ml of sample in 5 centrifugetube from respective flasks.Centrifuge all the tubes at 1500 rpm for 10 mins.Discard the supernatant and note their weight.Perform these steps after every 24hrs.

4. Haemocytometer Count: Take Haemocytometer slide and load the sample on it.Observe the slide under 45X and count the number of cells in each WBC chamber. Repeat above steps for every sample and record the observations.Perform these steps after every 24hrs.

5. Standard Plate count: Prepare 5 Sabouraud's agar butts and inoculate 1ml of each sample in each tube.Pour the butt into sterile petriplates.Incubate the plates at 28^0 c for 48hrs.Count the number of colonies in each plate and record the

results.Perform these steps after every 24 hrs.

6. Determination of protein content by Biuret method:

i. **Procedure for Preparation of the Biuret reagent:** Dissolve 1.5g copper (II) sulphate pentahydrate and 6g sodium potassium tartrate in 500ml water. Add 300ml 10% (w/v) NaOH and make the volume to 1 litre with water. Add 1g potassium iodide to inhibit the reduction of copper. Store in a plastic container in the dark. Discard if any black or reddish precipitate is observed (Gornell, 1949).

ii. **Protocol for Biuret Method:** Take 7 tubes and label them as Blank and 1 to 6. Make dilutions of Egg albumin standards. Add 2 ml of 3N NaOH reagent to all the seven test tubes. Mix well. Keep in boiling water bath for 10 minutes. Cool the tubes properly. Add 2 ml of Biuret reagent in all tubes and centrifuge it for 5 min at 3000 rpm. Resuspend the supernatant into quvettes, and record the absorbance with a spectrophotometer at 545nm.First, take the absorbance (OD) of Blank and make it zero. Take the OD of all the tubes (No. 1-6). Wash the quvettes each time after taking the reading.

7. Determination of glucose content by DNSA method:

i. **Procedure for preparation of DNSA reagent:** Prepare 20mL of 2N NaOH. Weigh 1 g DNSA and dissolve in 20mL NaOH with the help of a magnetic stirrer. Weigh 30g of sodium potassium tartarate and dissolve in 50mLH_2O. Slowly pour sodium potassium tartarate solution in the DNSA and NaOH solution and made the volume up to 100mL (Note: Wait for the two to mix properly). Decant the contents in a brown bottle. Filter if necessary.

ii. **Protocol for DNSA method:** Take 7 tubes and label them as Blank and 1 to 6. Make dilutions of glucose standards. Add 0.5 ml of DNSA reagent to all the seven test tubes. Mix well. Keep in boiling water bath for 15 minutes. After cooling to room temperature in a cold water bath, record the absorbance with a spectrophotometer at 540nm.First, take the absorbance (OD) of Blank and make it zero. Take the OD of all the tubes (No. 1-6). Wash the quvettes each time after taking the reading.

RESULTS

Table1: Wet weight, Haemocytometer count and Standard plate count

Sample	**Wet Weight**			**Haemocytometer Count**			**Standard Plate count**		
	After 24hrs	After 48hrs	After 72hrs	After 24hrs	After 48hrs	After 72hrs	After 24hrs	After 48hrs	After 72hrs
Uninoculated whey	0.07g	0.10g	0.19g	0	0	0	0	**0**	0
Whey+Yeast	0.78g	0.86g	0.2g	10^4 Cells/ml	10^6Cells/ml	10^2 Cells/ml	10^3 CFU/ml	10^5 CFU/ml	10^1 CFU/ml
Whey+peptone +yeast	0.84g	0.92g	0.68g	10^6 Cells/ml	10^8Cells/ml	10^4 Cells/ml	10^4 CFU/ml	10^6 CFU/ml	10^3 CFU/ml
Whey+Yeast extract+ Yeast	0.76g	0.81g	0.15g	10^2 Cells/ml	10^4Cells/ml	10^1 Cells/ml	10^2 CFU/ml	10^4 CFU/ml	10^1 CFU/ml
Whey+Casein+ Yeast	0.98g	1.28g	0.23g	10^7Cells/ml	10^{10} Cells/ml	10^2 Cells/ml	10^7 CFU/ml	10^{10} CFU/ml	10^4 CFU/ml

Table-2: Biuret Method

Tube no.	**Conc. Of stock (ml)**	**Vol. of diluent (ml)**	**Vol. of stock (ml)**	**Total volume (ml)**	**Vol. of 3N NaOH (ml)**	**Incubate all tubes in boiling water bath for 10 mins. Cool the tubes properly.**	**Vol. of 2.5% $CuSo_4$ (ml)**	**Incubate all the tubesat room temperature. Centrifuge all the tubesat 3000 rpm for 5 mins.**	**O.D at 545 nm**
1	Blank	4	-	4	2		2		0.0
2	1	3.2	0.8	4	2		2		0.11
3	2	2.4	1.6	4	2		2		0.14
4	3	1.6	2.4	4	2		2		0.20
5	4	0.8	3.2	4	2		2		0.24
6	5	0	4	4	2		2		0.28
7	Unknown	-	4 ml of unknown	4	2		2		2.25

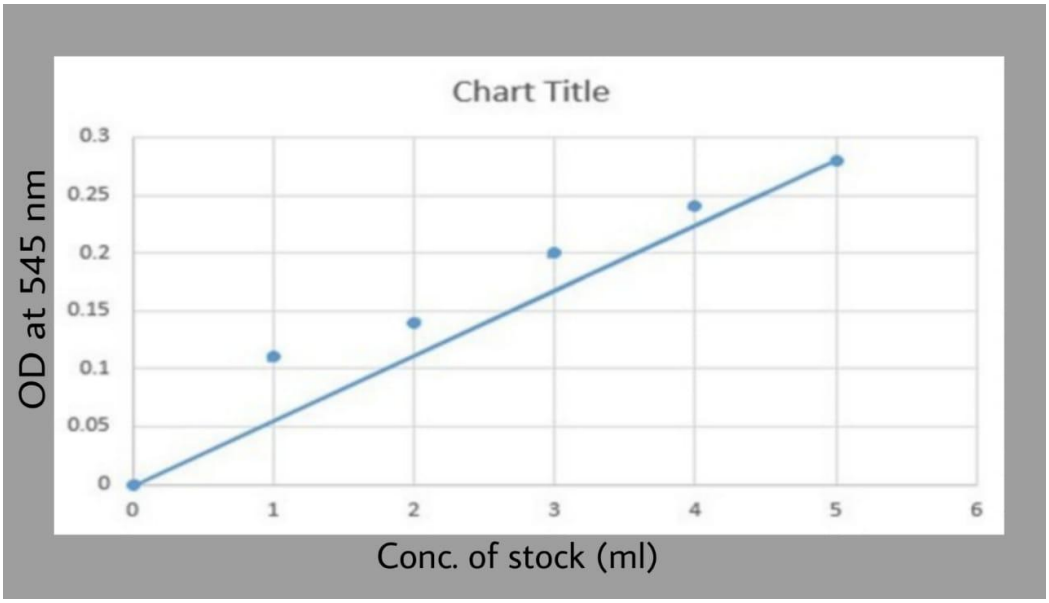

Figure-1: Determination of protein content by Biuret method

Table-5: DNSA Method

Tube No.	Conc. of stock	Vol. of diluents (ml)	Vol. of stock (ml)	Total vol. (ml)	Vol. of DNSA (ml)	Incubate all the tubes in the boiling water bath for 10 mins and then cool all the tubes properly.	Vol. of D/W (ml)	O.D at 545nm
1	Blank	1.0	-	1	0.5		8.5	0.0
2	200	0.8	0.2	1	0.5		8.5	0.05
3	400	0.6	0.4	1	0.5		8.5	0.07
4	600	0.4	0.6	1	0.5		8.5	0.11
5	800	0.2	0.8	1	0.5		8.5	0.16
6	1000	0.0	1.0	1	0.5		8.5	0.48

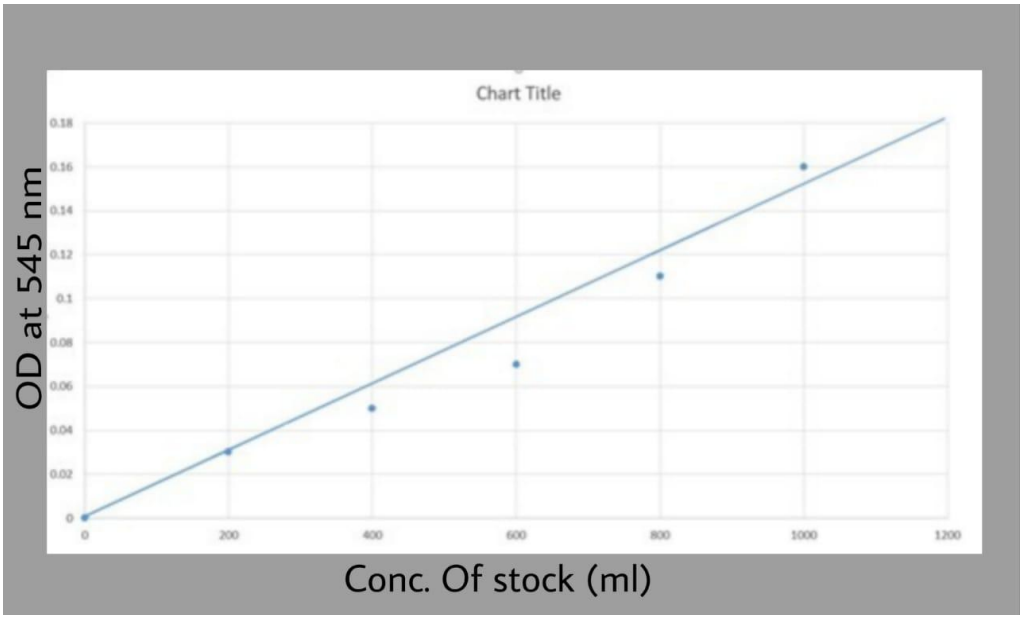

Figure-2: Determination of glucose content by DNSA method

Figure-3: Inoculation of Baker's Yeast into Cheese Whey

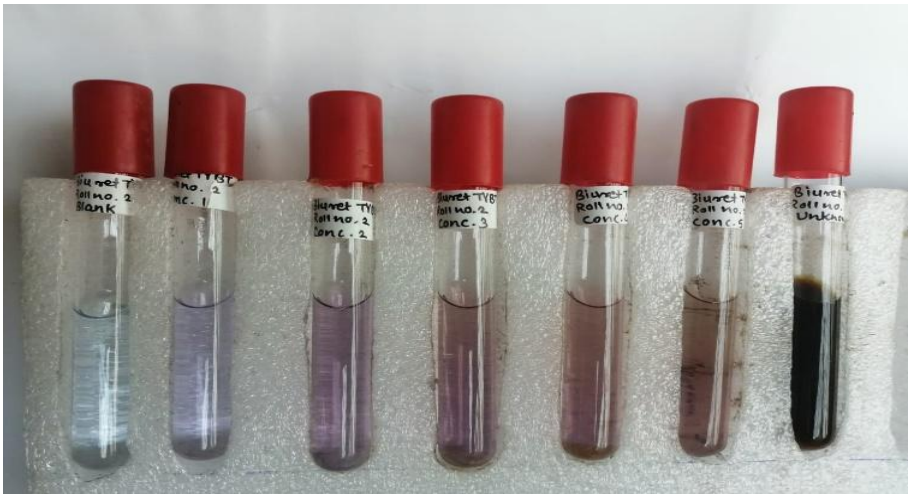

Figure-4: Determination of protein content by Biuret method

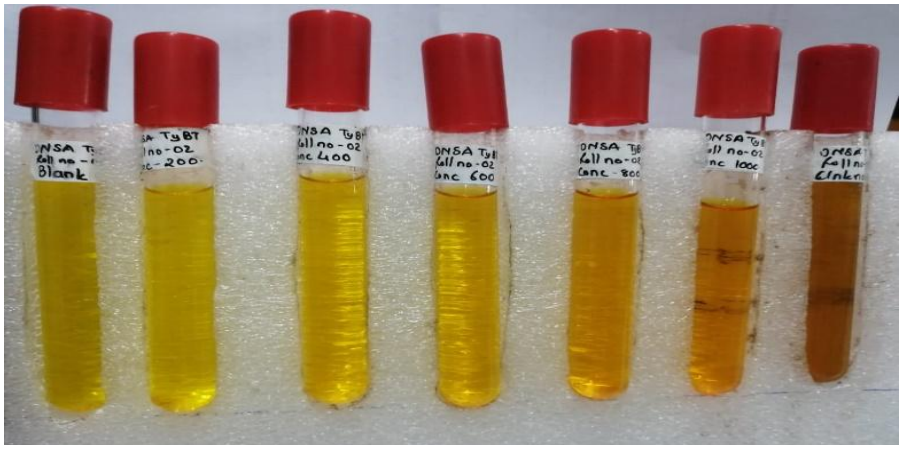

Figure-5: Determination of glucose content by DNSA method

DISCUSSION

In this experiment five different sets were run to compare the production of yeast on cheese whey using different substrates such as whey, whey+peptone, whey+yeast extract and whey+casein. Uninoculated whey is considered as blank. Baker's yeast (*Saccharomyces cerevisiae)* is inoculated into all flask except blank. Growth of cells were calculated by wet weight, Haemocytometer count and standard plate count after every 24 hrs. It was found that maximum growth was obtained in whey+casein followed by whey+peptone, whey+yeast and whey+yeast extract. As the maximum growth was found in whey+casein it is a good substrate for production of yeast. The amount of protein content in cheese whey was calculated by Biuret method and it was found to be 40.8 mg/ml. The amount of sugar content was calculated by DNSA method and it was found to be 3.4 mg/ml. As the number of cells, after 48 hrs are decreasing indicate that all the nutrients are consumed by the yeast cells therefore batch fermentation technique should be used and media should be changed after every 48hrs for better cell growth.Cheese whey disposal necessitates a substantial amount of industrial capital to avoid major environmental problems due to its high

biological and chemical oxygen demand, primarily due to high lactose content. As a result, finding a solution to the whey disposal problem is evident, and the "biorefinery," in which residual biomass, such as whey, is used to produce a variety of products such as animal feed, bulk and fine chemicals, could be the key to success.

CONCLUSION

Whey generated in cheese manufacturing processes serious environmental issues that limit process profitability. The goal of this research is to improve a fermentation method that uses whey as a growth medium and carbon source. To overcome the limitations in the use of lactose and to use cheese whey as a raw material for the development of *S. cerevisiae*we used an externally added substrates such as peptone, yeast extract and casein. Baker's yeast (*Saccharomyces cerevisiae)* is inoculated into all flask except blank. Growth of cells were calculated by wet weight, Haemocytometer count and standard plate count After every 24 hrs. It was found that maximum growth was obtained in whey + casein followed by whey + peptone, whey + yeast and whey + yeast extract. As the maximum growth was found in whey + casein it is a good substrate for production of yeast. The amount of protein content in cheese whey was calculated by Biuret method and it was found to be 40.8 mg/ml. The amount of sugar content was calculated by DNSA method and it was found to be 3.4 mg/ml. Therefore, cheese whey is an excellent growth medium and contains all necessary nutrients required for the growth of Baker's yeast**.** *S. cerevisiae* large scale fermentation, employment can be done bylimiting economical factor and yeastused in preparation of bread, alcoholic beverages, probiotics; by using cheap source of substrate ultimately lower the price of final product.

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REFERENCES

1. Boutrou, R., Sepulchre, A., Pitel, G., Durier, C., Vassal, L., Gripon, J. C., & Monnet, V. (1998). Lactococcal lysis and curd proteolysis: two predictable events important for the development of cheese flavour. *International Dairy Journal*, *8*(7), 609-616.
2. Christensen, J. E., Dudley, E. G., Pederson, J. A., & Steele, J. L. (1999). Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie van Leeuwenhoek*, *76*(1), 217-246.
3. Fox, P. F., Wallace, J. M., Morgan, S., Lynch, C. M., Niland, E. J., & Tobin, J. (1996). Acceleration of cheese ripening. *Antonie van Leeuwenhoek*, *70*(2), 271-297.
4. Ghaly A.E., Rushton D.G., Mahmoud N.S., 2007, Potential air and groundwater pollution from continuous highland application of cheese whey, American Journal of Applied Sciences, 4, 619-627.
5. González Siso M.I., 1996, The biotechnological utilization of cheese whey: A review, Bioresource Technology,57 (1), 1-11.
6. Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. Journal of biological chemistry, 177(2), 751-766.
7. Kunji, E. R., Mierau, I., Hagting, A., Poolman, B., &Konings, W. N. (1996). The proteotytic systems of lactic acid bacteria. *Antonie van Leeuwenhoek*, *70*(2), 187-221.
8. Mierau, I., Kunji, E. R., Leenhouts, K. J., Hellendoorn, M. A., Haandrikman, A. J., Poolman, B., ... &Kok, J. (1996). Multiple-peptidase mutants of Lactococcus lactis are severely

impaired in their ability to grow in milk. *Journal of bacteriology*, *178*(10), 2794-2803.

9. Mollea C., Marmo L., Bosco F., 2013, Valorisation of Cheese Whey, a By-Product from the Dairy Industry,Food Industry, Dr.InnocenzoMuzzalupo (Ed.), InTech, DOI,10.5772/53159.
10. Pisano, I., Agrimiab, G., Grossoa, G., Menaa, M. C., Ricciab, M. A., & Palmieriabc, L. (2015). Improved Saccharomyces cerevisiae growth on cheese whey by controlling enzymatic lactose hydrolysis. CHEMICAL ENGINEERING, 43.
11. Sansonetti S., Curcio S., Calabrò V., Iorio G., 2010, Evaluation of the parameters effects on the bioethanolproduction process from Ricotta cheese whey, Chemical Engineering Transactions 20, 13-18.