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Comparative Study of Biomass Yield (Single Cell Protein Production) Of Saccharomyces Cerevisiae and Lactobacillus Sporogenes over the Same Incubation Period from Dairy Waste Water

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Abstract: *The dairy industry, revolving around milk processing, generates large volumes of industrial effluents. These effluents can be nutritious and high in organic matter, with the main component being lactose, and thus problematic to handle for municipal wastewater treatment plants. Consequently around 50% of dairy wastes are discharged to receiving water-bodies, with potential environmental consequences.*

The dairy industry involves processing raw milk into products such as consumer milk, butter, cheese, yogurt, condensed milk, dried milk (milk powder), and ice cream, using processes such as chilling, pasteurization, and homogenization. The typical byproducts of milk are buttermilk, whey, and their derivatives. The effluents are generated from milk processing through milk spillage, drippings, washing of cans, tankers bottles, utensil, and equipment's and floors. The dairy industry generates on an average 2.53litres of wastewater per litre of milk processed. Generally this wastewater contains large quantities of fat, casein, lactose, and inorganic salts, besides detergents, sanitizers etc. used for washing. These all contribute largely towards their high biological oxygen demand (BOD), chemical oxygen demand (COD) and oil and grease much higher than the permissible limits.

The aim of this study was to produce Single Cell Protein (SCP) i.e. Biomass and removal of COD from dairy wastewater. The decrease in lactose i.e. Organic load and increase in biomass (SCP) occurred in parallel &growth rate also increased simultaneously with increasing lactose consumption rate. Finally, cost effective SCP process and product can be performed in an industrial scale and the product can be consumed instead of expensive protein in market.

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I. Introduction

Global production of liquid whey from cheese and casein amounted to 186 million MT in 2008 with an annual average growth of approximately 2% between 2002 and 2008 **(USDEC, 2003)** Today, the annual global increase of whey production is equivalent to 2%, which is parallel to annual increase in milk production**(Bozanic *et al.* 2014**). During manufacture of cheese products, approximately 80 million tons of sweet and sour whey are produced annually in India. Only a very small proportion of this huge amount of whey is used for animal nutrition, and roughly 50% of the whey produced is used to formulate products, i.e., food and feedstuffs. The remainder is treated as waste. It is sometimes dumped into surface water or sprayed onto fields, thus causing damage to local ecosystems. Because of high moisture content, it is not economic to transport the whey for drying. In addition, the energy costs of drying are extremely high. As a result, in view of the current sales prices, the manufacture of whey powder is not a profitable activity. Whey may be used as a valuable raw material for a wide range of applications in the food, pharmaceutical and biogas industries **(Panesar and Kennedy 2012**). However, the amount of whey processed worldwide is only about half of the annual global whey output.An alternative to the traditional uses of whey is the production of single-cell protein (SCP) (**Farhoodi Somaye *et al.,* 2008**). During manufacture of SCP, whey is inoculated with appropriate bacteria and yeasts (**Nasseri*et al,* 2011**). The lactose and lactate contents of whey provide nutrients for these microorganisms to grow properly and produce proteins. The finished product, which has a protein content of approximately 50%, is of high biological value (87%).

SCP refers to the dried cells of microorganisms. After proper pre-treatment, it is utilized in both human and animal nutrition as a food and feed supplement **(Schingoethe1976**). Starting in the 1950s, very intensive research activity had been focused on the issues of SCP production but then it largely slowed down due to

economic reasons. However, because of the efforts made worldwide to reduce environmental load, the question of whey utilization has recently become a high-priority research topic again. Dairy industry seeks cost-effective treatment technologies to remove organic matter and nitrogen from food processing wastewater containing high levels of suspended solids and nitrogenous compounds **(Gadgil 1978).** Increasing concern about pollution that occurs from agricultural and industrial wastes has stimulated interest in converting waste materials into commercially valuable products, especially SCP. These waste products can be converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms. Algae, fungi & bacteria are the chief sources of microbial protein that can be utilized as SCP . Technically, SCP is the manufacture of cell mass using microorganisms by culturing on abundantly available wastes.

The primary purpose of this research was to find cultures suitable for use in commercial production of SCP from whey. The interest in using dairy wastewater as substrate for SCP production has increased concomitantly.

Dairy wastes, due to their composition, volumes and fluctuating flow rates, represent a problem for municipal wastewater treatment plants. As a result it has been common practice to discharge the waste into receiving waters. Thus wasting potential resources (e.g. lactose) and polluting water-bodies at the same time. comparatively more than that of dairy waste water. The total amount of protein, carbohydrate and fats in untreated dairy waste water were found to be suitable according to the requirements for the growth of SCP. Lactose is necessary constituent for the biomass production. Lactose act as substrate for the SCP production.

II. Methodology

1) **Site of sample collection Saras**

Dairy:

The initial handling capacity of this dairy plant was 1.5 Lakh litres per day. It sells milk and milk products through a network of over 5013 retail outlets spread over Jaipur city and nearby 100 towns. Over the years, significant growth has been made in all fields of Jaipur Dairy i.e. procurement, processing and production of various milk and milk products.

Its marketing is done under the brand name of SARAS.

2) **Sampling of dairy waste water**

For present study, waste water was collected before and after treatment from the Jaipur Dairy, Rajasthan. Samples were collected in clean plastic sampling bottles, were transferred to laboratory and stored at $4^{o}C$

3**) Production of SCP**

Inoculum preparation

Two cultures *Saccharomyces cerevisiae* and *Lactobacillus sporogenes* were used as inoculums for production of SCP. *Saccharomyces cerevisiae* was prepared from YPD (yeast peptone dextrose) agar incubated at 25^{0} C for 72 hours and Lactobacillus sporogenes was prepared from Nutrient agar incubated at $37^{0}C$ for 48 hours were used as inoculums.

Shake flask fermentation: The cultures were then scooped from the surfaces of the agar into 500 ml autoclaved Erlenmeyer flask containing 250 ml untreated dairy waste water. The Erlenmeyer flask with *Saccharomyces cerevisiae* inoculation was incubated at $30^{0}C$ with constant shaking of 250 rpm for following incubation periods that were 5, 10, 15 and 20 days. The Erlenmeyer flask with Lactobacillus sporogenes inoculation was incubated at $37^{0}C$ with constant shaking of 120rpm for following incubation periods that were 5, 10, 15 and 20 days. Biomass obtained after filtration was kept at $60^{0}C$ temperature for 24 hours to obtain powder form. Dry cell yield was measured for both isolates biomass.

III. Observation

Production of SCP

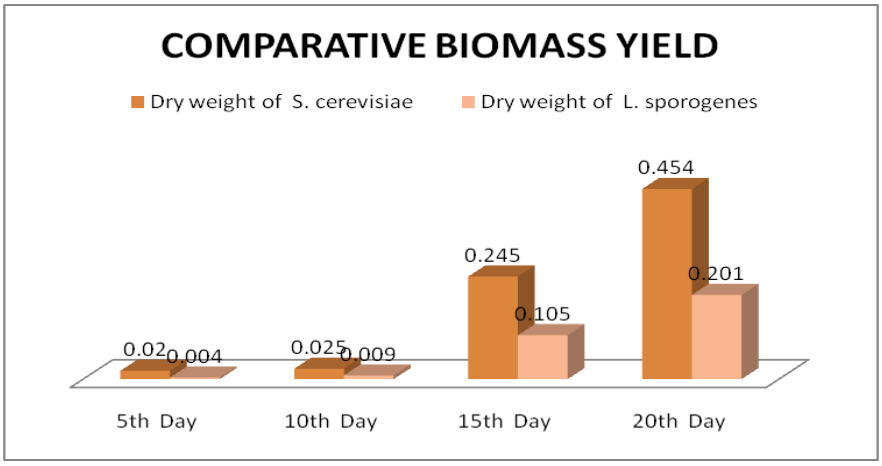
The main component of dairy wastewater is lactose which is also largely responsible for the high BOD and COD. These isolates utilized the lactose and reduced the high organic load in Dairy wastewater that dramatically enhances biomass production. Both isolates showed good biomass production in flask which is significant.

Values obtained are indicated below tables. It is found that there is increase in protein concentration and biomass for observed periods of 20 days at intervals of 5 days.

Comparative study showed that *Saccharomyces cerevisiae* gives more biomass yield as compared with *Lactobacillus sporogenes* with same incubation period.

Result of biomass yield (g/L)	5th Day	10th Day	15th Day	20th Day
Dry weight of *S. cerevisiae*	0.02	0.025	0.245	0.454
Dry weight of *Lsporogenes*	0.004	0.009	0.105	0.201

Table : Result of biomass yield



Graph : Showing comparative biomass yield over the same incubation period.

IV. Results and Conclusion

Dairy waste can act as substrate for the growth of culture and production of biomass. This microbial biomass can be further used for the production of edible unicellular organisms- SCP. On one hand, organic load decreases and on the other hand biomass yield increases that will lead to the production of SCP.

The main composition of dairy waste water is lactose, lactose being largely responsible for high BOD and COD. *S.cerevisiae* and *L. sporogenes* utilized the lactose and remove the high organic load in dairy wastewater. These species therefore could be used as an attractive alternative for removal of dairy wastewater and obtaining a valuable biomass yield. The decrease in lactose and increase in biomass occurred in parallel and growth rate also increased simultaneously with increasing lactose consumption rate. The phenomenon justifies our assumption that lactose is the growth controlling substrate. The culture biomass produced during the treatment process can be used as a rich source of protein supplement in animal feeds.

Finally the cost effective SCP process can be performed in an industrial scale and the product can be consumed instead of expensive proteins present in the market. In this research study the comparative study showed that *S. cerevisiae* yield more biomass as compared to *L.sporogenes.*

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