Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2011, 1 (3):209-215

ISSN: 2248 –9215

Production of Single cell protein and removal of 'COD' from dairy waste water

Jitendra Kumar Singh*[1], Rahul L. Meshram[2] and D. S. Ramteke[3]

[1]*Institute of Environment & Development Studies, Bundelkhand University, Jhansi. (U.P)*
[2]*Shri Shivaji College, Akola (M.H)*
[3]*National Environment & Engineering Research Institute (NEERI), Nagpur (M.H).*

ABSTRACT

The aim of study was to produce Single cell protein (SCP) i.e. Biomass and removal 0f 'COD' from dairy waste water. Dairy waste water was collected and analyzed for Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Total suspended solids (TSS). The main composition of dairy waste water is Lactose (about 70%), lactose being largely responsible for the high organic load in dairy waste water. Thus, dairy waste water is particularly suitable for the production of SCP, using lactose utilizing microorganisms. Saccharomyces cerevisiae & Humicola species were evaluated for their ability to grow and produce biomass and reduce the organic load of the Production of dairy waste water. Saccharomyces cerevisiae & Humicola species were able to reduce COD by about 62% & 93% respectively, with continuous biomass production. 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.

Keywords: Dairy waste water, Single cell protein (SCP), COD, *Saccharomyces cerevisiae* & *Humicola* species.

INTRODUCTION

Single-cell protein (SCP) refers to the dried cells of microorganisms. SCPs are used as protein sources in human foods or animal feeds. With rapid industrialization and development, problems of pollution, poverty and population is increasing at a very fast rate and the demand on the earth. Water resources are most often affected by industrial pollution. The worldwide dairy industry generates over 80 million tons of whey each year. Because of enhanced industrial activities and rising standard of living, our environment is now polluted by industrial wastes, especially liquid ones. Among the liquid industrial wastes, dairy effluents pose a serious problem to our

environment. Dairy effluent has high organic loads as milk is its basic constituent with high levels of chemical oxygen demand, biological oxygen demand, oil & grease and nitrogen and phosphorous content (Braio & Granhem Taveres, 2007). Because of its high organic content with high BOD, dairy waste water dumped directly to the environment is causing serious contamination problems. 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 (Leman et al., 1990). These waste products can be converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms (Atalo and Gashe, 1993). Algae, fungi and bacteria are the chief sources of microbial protein that can be utilized as SCP (Anupama & Ravindra, 2000). Technically, SCP is the manufacture of cell mass using microorganisms by culturing on abundantly available wastes. Several agro-industrial wastes have been used to produce single cell protein (SCP) for rumen and poultry feed (Haddadin et al., 1999; Paul et al., 2002). Usually, the substrates advocated for SCP production have been carbohydrate-rich waste materials, such as agricultural wastes (Imrie & Righelato, 1976), The main composition of dairy wastewater is lactose (about 70%), lactose being largely responsible for the high BOD and COD. Thus, dairy wastewater is particularly suitable for the production of SCP using lactose-utilizing microorganisms. Much interest has been focused on the potential of converting soy milk wastes, potato effluents, sugarcane bagasse, orange peels, shrimp-shell wastes, kimchi production wastes or forestry wastes (e.g. wood hydrolysates) to single cell protein (SCP) (Cheung, 1997; Choi, & Park, 1999; El-Nawwi & El-Kader, 1996; Ferrer, Paez, Marmol, Ramones, Garcia, & Forster, 1996; Parajo, Santos, Dominguez, & Va´ zquez, 1995; Schugerl & Rosen, 1997; Ziino, Lo Curto, Salvo, Signorino, Chiofalo, & Giuffrida, 1999). The yeasts have the advantages of large size, low nucleic acid content, long history of use as food and ability to grow at low pH (Mitchel, 1974). The use of filamentous fungi is advantageous over bacterial treatments. These advantages include easy separation of fungal biomass, higher rate of COD reduction and use of fungal protein as feed supplement to poultry and pigs (Jin et al., 1999). The interest in industrial and municipal wastes as substrate for SCP production has increased concomitantly.

MATERIALS AND METHODS

2.1. Collection of wastewater and its characteristics

Dairy industry wastewater was collected in plastic containers from Government milk scheme, located at Nagpur (Maharashtra) and they stored in refrigerator at 4°C temperature. The characteristics Dairy Wastewater was determined (Table 1).

The five day BOD of wastewater was determined by using the azide modification method (American Public Health Association, 1995). Chemical oxygen demand (COD) was estimated by the open reflux method described in the standard methods for examination of water and wastewater (APHA, 1995). Total suspended solids (TSS) were measured by filtration of a sample through a pre-weighed filter paper followed by drying at 105^0C for 24 h to constant weight.

Table 1: Characteristics of Dairy Wastewater

Components	Concentration
Colour	Milky
Odour	Unpleasant
pH	4.06
Conductivity	380
Temperature	28 ^{0}c
Turbidity	700 NTU
Chloride	22.29 mg/l
Total Suspended Solids (TSS)	1196 mg/l
Total Dissolved Solid (TDS)	512 mg/l
Biological Oxygen Demand (BOD)	3070 mg/l
Chemical Oxygen Demand (COD)	5760 mg/l
Oil & Grease	136 mg/l

2.2. Micro-organism

Humicola sp. (ISO1) and Saccharomyces cerevisiae (ISO2) was isolated from dairy effluent and On the basis of Morphological Characteristics & Biochemical characteristics of two Genera Saccharomyces & Humicola were identified by using the methods and identification keys for fungi (Reed G. Et al., 1991) and Hand book of soil fungi (I.K. International Pvt. Ltd. 2006) respectively. This culture was grown on Potato-dextrose agar (PDA) slants for 48 h at 37°C and then stored at 4°C with subculturing every 2 months.

2.3. Inoculum preparation

Isolated two cultures prepared from 4 days growth on PDA slants incubated at 37^{0}c was used as inoculums. The cultures were then scooped from the surface of the agar in to 250 ml autoclaved dairy waste water in 500 ml Erlenmeyer flask. Then capped with cotton and the Erlenmeyer flask was incubated at 25^{0}c with constant shaking of 120rpm.

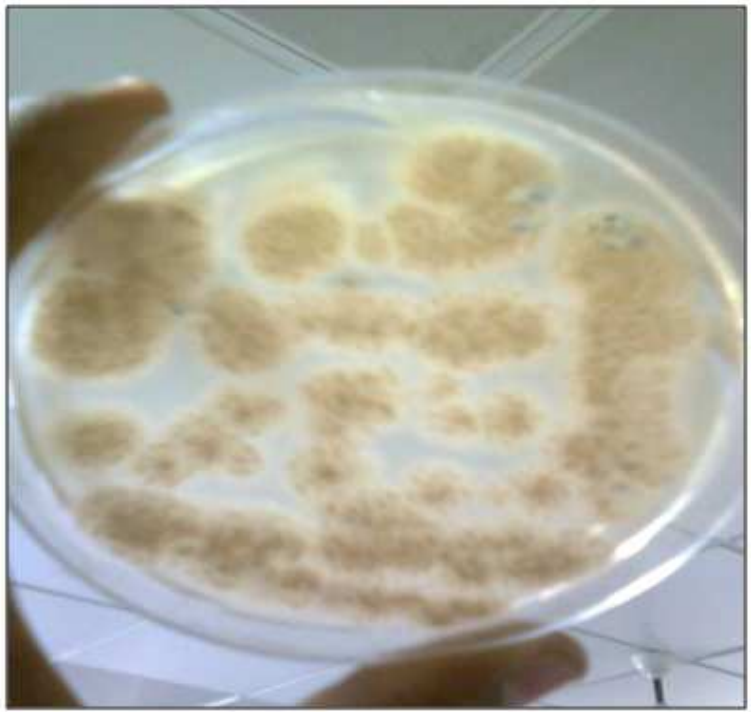
2.4 Characterization of the Isolated Microorganisms

2.4.1 Morphological Characteristics of the Isolates

Colonies of Isolate **ISO2** in medium grow rapidly and mature in 3 days. They are flat, smooth, moist, glistening or dull, and cream to tannish cream in color, Cell buds (Blastoconidia) are observed. They are unicellular, globose, and ellipsoid to elongate in shape. Multilateral (multipolar) budding is typical, hyphae are absent. They multiply as single cells that divide by budding & grow as simple irregular filaments (mycelium). In sexual reproduction they produced haploid ascospores. These ascospores are globose and located in asci. Each ascus contains 1-4 ascospores.

Whereas, the colonies of **ISO1** in medium grow & mature in 7 days incubation, they are effuse colonies, cottony, sometimes funiculose, at first white, later grey, brown with age. Mycelium superficial and immersed, stroma, setae and hyphopodia absent, Conidiophores are micro or semi-macronematous, unbranched or irregularly branched, straight or flexuous, colourless to pale golden brown, smooth. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical, doliform, pyriform,or infundibuliform.They produced conidia (aleuro conidia) solitary, dry, acrogenous, simple, typically spherical, occasionally abovoid or pyriform, pale to midgolden brown, 0-septate, smooth.

Humicola species



2.4.2 Biochemical Characteristics of the Isolates

Table 2: Sugar Fermentation Test

Isolates	Glucose	Lactose	Sucrose	Mannitol
	A/G	A/G	A/G	A/G
ISO1	+/-	+/-	+/-	+/-
ISO2	+/-	+/-	+/-	+/-

RESULTS AND DISCUSSION

3.1 Protein Estimation

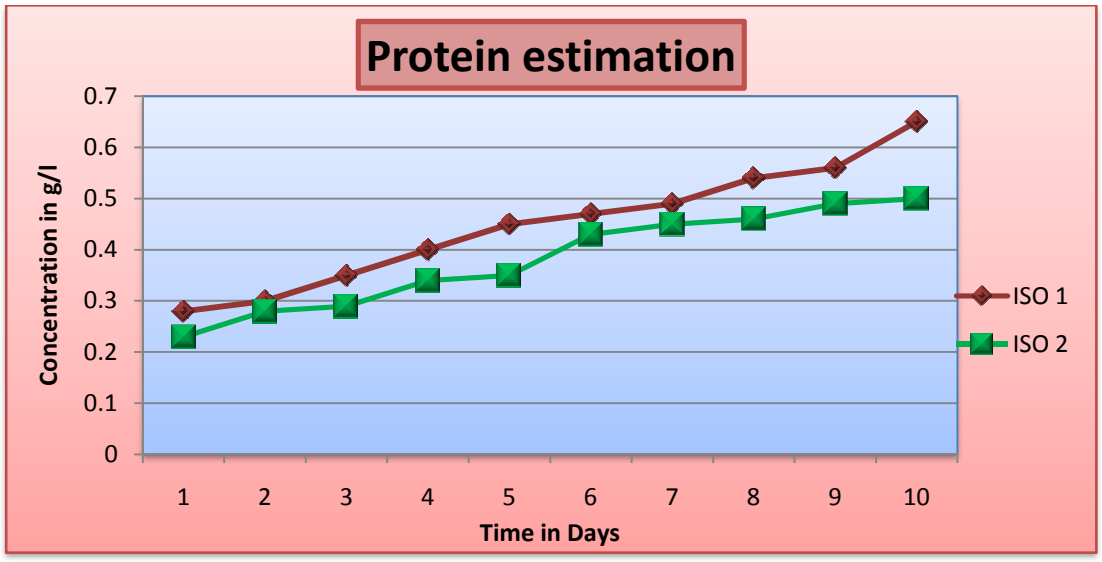


Figure 1: Protein Estimation

Table 3

Time in Days		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Protein g/l	ISO1	0.28	0.3	0.35	0.4	0.45	0.47	0.49	0.54	0.56	0.65
	ISO2	0.23	0.28	0.29	0.34	0.35	0.43	0.45	0.46	0.49	0.50

3.2 Biomass Estimation

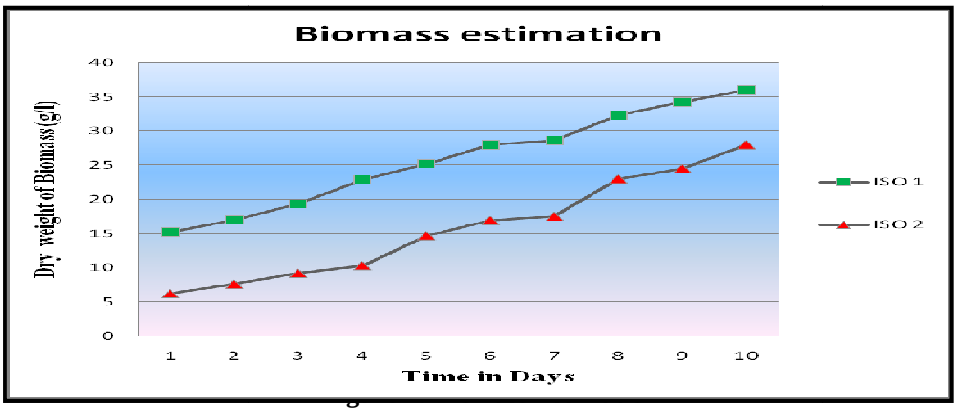


Table 4

Time in Days		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Dry weight of Biomass (g/l)	ISO1	15.2	17.0	19.3	22.9	25.2	28.0	28.6	32.3	34.2	36.0
	ISO2	6.2	7.6	9.2	10.3	14.7	16.9	17.5	23.0	24.5	28.0

3.3 Removal of COD

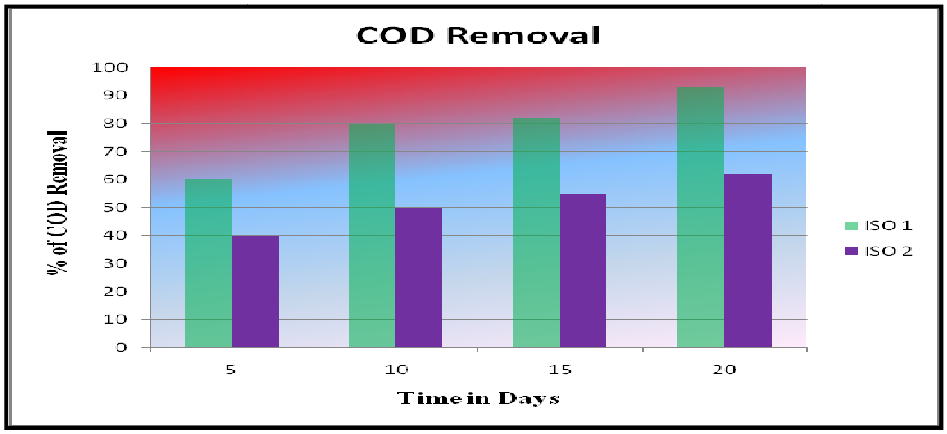


Figure 3: COD Removal

Table 5

Culture Species	COD Removed			
	5th Day	10th Day	15th Day	20th Day
ISO 1	60 %	80 %	82 %	93 %
ISO 2	40 %	50 %	55 %	62 %

Protein concentration and biomass was analysed simultaneously with COD. Values obtained are indicated in above tables. It is found that there is constant increase in protein concentration and

biomass with removal of COD for observed period of 10 days for protein concentration and biomass, where 20 days for COD at intervals of 5 days. (Fig 1, 2 & 3).

ISO1 showed initial value 0.28 g/l of protein, 15.2 g/l of biomass on 1st day of incubation. A final value for the same on 10th day was 0.65 g/l and 36.0 g/l respectively. 0.50 g/l of protein and 28.0 g/l of biomass was reported on 10th day as compared to 0.23 g/l protein and 6.2 g/l biomass on 1st day for ISO2.

The study of COD removal from dairy wastewater (Garrido et al., 2000) indicated that, COD removal efficiency was achieved between 80 to 90%, similar to our study. ISO2 is able to reduce COD with high efficiency with value of 93% than ISO1 with value of 62% was observed period of 20 days. The highest COD removal efficiency was more than (90%) and the best sludge settling properties for the milk factory wastewater were obtained at high sludge age (20 days) and aerated period of 6 hrs, showing a good concordance with the results presented by the study of Flapper and Ashbolt (1999).

CONCLUSION

Morphological and biochemical characteristic study showed that culture obtained from dairy wastewater is ***Saccharomyces cerevisiae*** and ***Humicola*** Species. Moreover, when tested at laboratory scale ***Saccharomyces cerevisiae*** is reduced organic load (COD) by 62% after twenty days of incubation with continuous biomass production. Species of ***Humicola*** fungus, which is another isolate, able to reduce COD by 93% compared over the same dairy waste before introduction of Humicola**.** We also estimated lactose fermentation, both these isolates capable to ferment lactose. The main composition of dairy wastewater is lactose, lactose being largely responsible for the high BOD and COD. These isolates 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 waste water COD 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, 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.'

REFERENCES

[1]. American Public Health Association, **1995**. Standard Methods for the Examination of Water and Wastewater, 19th ed., Washington, DC APHA, pp. 9.10–9.15.
[2]. Anupama X & Ravindra P, Biotechnology Advances, **2000**, 18, 459–479.
[3]. Atalo K, Gashe B.A, *Biotechnol. Lett*., **1993**,15, 1151–1156.
[4]. Braio V.B and C.R. Granhem Taveres, *Journal of Chemical Engineering*, **2007**, 24(4): 487-497.
[5]. Cheung P. C.-K, *Food Chemistry*, **1997**, 60, 61–67.
[6]. Choi M. H & Park Y. H, *Bioresource Technology*, **1999**, 70, 231–236.
[7]. El-Nawwi S. A., & Anal Abd, El-Kader *Biomass and Bioenergy*, **1996**, 11, 361–364.
[8]. Ferrer J, Paez G, Marmol Z, Ramones E, Garcia H, & Forster C. F, *Bioresource Technology*, **1996**, 57, 55–60.

[9]. Flapper T G, Ashbolt N J (**1999**). Treating industrial wastewater using a SBR. Proceeding of the AWWA 18TH Federal Convention, April 1999.
[10]. Gadgil J.S, Hanumanulu V and Subrahmanyam. P.V.R, (**1978**): Dairy effluent treatment in India. Bulletin of IDF 104, 207
[11]. Garrido J M, Omil F, Arrojo B, Mendez R, Lema J M (**2000**), Proceedings of the 2nd International symposium on Sequencing Batch Reactor Technology, pp 243-250.
[12]. Haddadin M.S, Abdulrahim S.M, Alkhawaldeh G.Y, Robibson R.K, *J. Chem. Technol. Biotechnol*, **1999**, 74, 613–618.
[13]. Imrie F. K. E. & Righelato R. C, (**1976**), G. G. Birch, K. J. Parker & J. T. Worgan, pp. 79-97. Applied Science, London.
[14]. Jin B, Leeuwen H.J.V, Patel B, Doelle H.W, Yu Q, *Process Biochem*., **1999**, 34, 59–65.
[15]. Leman J, Bednarski W, Grewal H.S, *Ind. J. Microbiol*., **1990**, 30, 13–28.
[16]. Mitchel R, (**1974**), Microorganisms as Food. An Introduction to Environmental Microbiology, Prentice-Hall, Englewood Cliffs, N J, pp. 297-308.
[17]. Nagamani A, Kunwar I.K. and Manoharachary C, (**2006**), Handbook of Soil Fungi. I. K. International Pvt. Ltd. New Delhi.
[18]. Parajo J. C, Santos V, Domı´nguez H, & Vaszquez M, *Food Chemistry*, **1995**, 53, 157–163.
[19]. Paul D, Mukhopadhyay R, Chatterjee B.P, Guha A.K, *Appl. Biochem. Biotechnol*., **2002**, 97, 209–218.
[20]. Reed G, Tilak WN, Yeast technology, Second edition, New York, **1991**.
[21]. Schugerl K, & Rosen W, *Process Biochemistry*, **1997**, 32(8), 705–714.
[22]. Ziino M, Lo Curto RB, Salvo F, Signorino D, Chiofalo B, & Giuffrida D, *Bioresource Technology*, **1999**, 67, 7–11.