IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 3, Issue 4 (Jul. – Aug. 2017), PP 73-78
www.iosrjournals.org

Characterization of Dairy Waste and its Utilisation as Substrate for Production of Single Cell Protein

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Abstract: *Globally, the dairy sector is one of the most important sectors of the world. The milk processing industry has been emerging rapidly during the last two decades due to enormous increase in the milk production and increase in demand for milk and milk products. 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 by-products 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 and floors. The dairy industry generate on an average 2.5-3.0 litres of wastewater per litre of milk processed.*
On other hand the increasing world deficiency of protein is becoming a major problem for human kind. India, although a developed nation, its major population is facing nutrition deficiency and food scarcity problems. In the face of such worldwide issues, single cell proteins derived from the waste organic products have been proved a very useful technology. Dried cells of bacteria, yeast, fungi and algae, which are rich in proteins and could be used as dietary supplements, are called Single Cell Proteins (SCP). The overarching goal of the study is to characterize physiochemical and biological properties of both untreated and treated dairy waste water. After complete characterization the raw waste is utilized for the single cell protein production .Two strains lactobacillus acidophilus and saccharomyces cerevisiae were inoculated on waste and biomass was produced by shake flask fermentation process.Dairy waste water appears as suitable substrate for SCP production.

Date of Submission: 11-07-2017 Date of acceptance: 25-08-2017

I. Introduction

The increasing deficiency of protein worldwide is becoming a major problem for human kind. Some of the underdeveloped countries like Algeria, Botswana, Nigeria, Madagascar etc., are facing major food and nutrition deficiency problems.India, being a developing nation, also faces the problem of nutrition deficiency and food scarcity due to its rapid growing population. In the face of such worldwide issues, Single Cell Proteins derived from waste organic products has proved to be a very useful technology. SCP provides an alternative and innovative way to successfully solve the global food problem (Najafpur, 2007).

Dried cells of bacteria, yeast, fungi and algae, which are rich in proteins and can be used as dietary supplements, are called Single Cell Proteins (SCP). Microbial protein or SCP have various benefits over animal and plant proteins in that, their requirement for growth is neither seasonal nor climate dependent and thus they can be produced all-round the year (Nasseri *et al*., 2011). Besides, it does not require a large expanse of land, has high protein content with wide amino acid spectrum, low fat content and higher protein carbohydrate ratio than forages (Schultz *et al*., 2005). It can be grown on waste and is environmental friendly as it helps in recycling waste.

The four microbial groups that can be used for SCP production are bacteria, yeasts, fungi and algae. *Cellulomonas, Alcaligenes, Spirulina, Chlorella, Trichoderma, Fusarium, Rhizopus, Candida, Saccharomyces, Methylophilus, Kluyveromyces, Aspergillus,* etc. are some of the common microorganisms that have been used as SCP.

Various substrates have been used as starter material for production of various types of Single Cell Protein such as orange peel residue, sweet orange residue, sugarcane residue, paper mill waste rice husk, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, grape waste, mango waste, etc.(Nigam ,2000 ; Bozakuk,2002 and Zubi,2005).

Dairy waste can also act as potential substrate for SCP production. Dairy waste are high in lactose, nitrogenous compounds, minerals and also contain small amount of vitamins (Moeine *et al*., 2004).Being world's largest milk producer, India accounts for more than 13% of world's total milk production. Its products are a major source of cheap and nutritious food to millions of people in India (Dairytech India, 2011). Dairy

Cooperatives account for the major share of processed liquid milk marketed in India. Milk is processed and marketed by 170 Milk Producers Cooperative Unions, which federate into 15 State Cooperative Milk Marketing Federations.

However, the waste water discharged from these industries is a major source of pollution affecting the ecosystem. The degradation of environment caused by industrial waste has adversely affected the living organism and agriculture (Anikwe *et al.,* 2006). Dairy industry is amongst the most polluting of the food industries in regard to its large water consumption. Dairy is thus one of the major industries causing water pollution (Chaiudhari and Dhoble, 2010). Considering the increased milk demand, the dairy industry in India is expected to grow rapidly. However, waste generation and related environmental problems have thus also assumed increased importance. Poorly treated waste water with high level of organic pollutants caused by poor design, operation or treatment systems creates major environmental problems when discharged to the surface land or water. Effluent treatment in industries, to meet the discharge standards mentioned by Central Pollution Control Board (CPCB), has always been a great problem for the industrialists. Every industry effluent treatment plant needs to treat the effluent for this purpose in their self-industry via effluent treatment plants. Before discharging the treated effluent on to the land or any surface water body the industries should meet the effluent discharge standard norms. In order to have proper processes in the ETP, characterization of waste water, treatability studies and planning of proper units and processes for effluent treatment is very much necessary.

Milk content and characteristics vary from one place to another .Also, the waste products from a particular dairy, its treatment process and remaining effluents and sludge differ from one milk processing plant to other. Therefore, for every large scale dairy industry, the waste has to be characterized to find its utility and suitability for its potential use as substrate for SCP production. Thus, the present study is aimed at finding the potential use of dairy waste for producing a protein rich product that will act as protein supplement for increasing worldwide population.

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 until use for analysis.

3)Physico-chemical analysis of both untreated and treated waste water:

For the present study the samples were collected from dairy industry at the sources were analysed for physicochemical parameters such as pH, temperature, salinity, conductivity, BOD (Biological Oxygen Demand) and total dissolved solids (TDS) by water analyser kit (Fig.2). Chemical Oxygen demand (COD) is done by the method as given in American Public Health Association (APHA) manual (1995). All these parameters were analysed within 24 hours.

4)Biological analysis of both untreated and treated waste water:

Total fat: The fact that lipids are soluble in organic solvents but insoluble in water provides us with a convenient method of separating the lipid components in foods from water soluble components such as protein ,carbohydrate and minerals. In fact solvent extraction method is most useful method of determination of total fat present in the sample. In this process one of the components of a mixture dissolves in a particular liquid and the other component is separated as a residue by filtration.

Total Protein: For the total protein estimation Lowry's method was used.The principle behind the Lowry's method lies in reactivity of peptide nitrogen with copper ions under alkaline conditions and subsequent reduction of Folin-Ciocalteay phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by copper –catalyzed oxidation of aromatic acids.

Carbohydrate: The Anthrone method, a colorimetric method was used to determine the concentration of the total sugars in a sample. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green colour. The sample was mixed with sulfuric acid and the anthrone reagent and then boiled until the reaction was completed. The solution was then allowed to cool and its absorbance was measured at 620 nm.This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid.

Lactose: For the presence of lactose Benedict's test was done using benedict`s reagent made by complexing Cu^{+2} (from Copper sulphate) ions with citric acid in a basic medium (Sodium Carbonate). Benedict's reagent was used to detect reducing sugars. It oxidize aldehydes or alpha hydroxyl ketones that cause disappearance of the bluish colour and formation of a white precipitate of coporous thiocyanate.

5. Pathogen detection
Untreated dairy waste was analysed for various common bovine pathogens.For this different selective media were used for the detection of the pathogens.

6. Production of SCP
Inoculum preparation
Two cultures ***Saccharomyces cerevisiae and Lactobacillus acidophilus***were used as inoculums for production of SCP. ***Saccharomyces cerevisiae*** was prepared from YPD(yeast peptone dextrose) agar incubated at 30^0C for 72 hours and ***Lactobacillus acidophilus*** was prepared from Nutrient agar incubated at 37^0C for 48 hours were used as inoculums.

Shake flask fermentation: The cultures were then scooped from the surfaces of the agar into500 ml autoclaved Erlenmeyer flask containing 250 ml untreated dairy waste water. The Erlenmeyer flask with ***Saccharomyces cerevisiae*** inoculation was incubated at 30^0c with constant shaking of 250 rpm for 7 days. The Erlenmeyer flask with ***Lactobacillus acidophilus*** inoculation was incubated at 37^0C with constant shaking of 120rpm for 7 days. Biomass obtained after filtration was kept at 60^0C temperature for 24 hours to obtain powder form. Dry cell yield was measured for both isolates biomass.

Characterization of the Microorganisms used as inoculums:
Morphological Characteristics of the Isolates
Colonies of ***Saccharomyces cerevisiae*** in medium grow rapidly. They are flat, smooth, moist, glistening or dull, and cream to tannish cream in colour, Blastoconidia were observed. They are unicellular, globose, and ellipsoid to elongate in shape. Multipolar budding was typical, hyphae were absent. They multiply as single cells that divide by budding & grow as simple irregular filaments /mycelium.

Lactobacillus acidophilus
Colonies of ***Lactobacillus acidophilus*** in medium grow rapidly. They are gram positive. They are long slender rods to short.

III. Observation

a) Physico-chemical analysis
pH: - It is a term used to express the intensity of the acid or alkaline condition of a solution. It is a way of expressing the hydrogen-ion concentration or the hydrogen-ion activity. Pure water is said to be neutral, with a pH close to 7.0 at 25 °C (77 °F). Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are said to be basic or alkaline. Though the pH is alkaline in fresh conditions, the waste becomes acidic due to decomposition of lactose into lactic acid under anaerobic conditions. pH of untreated dairy waste water was found to be 6.4 and pH of treated dairy waste water was found to be 7.4
Temperature: Pure water temperature is approximately 25 °C .Temperature of untreated wastewater was found to be between 28 to 30 °C and that of treated waste water was found to be between25 to 28 °C.
Turbidity: Colloidal and particulate components in the waste reflect light back to an observer. This is known as apparent colour. The concept of turbidity is closely related to this phenomenon. Milk wastes contain significant quantities of material that will result in turbidity of discharges. Turbidity of effluent discharge from SARAS dairy was found to be 25 NTU and treated dairy waste was found to be have 20 NTU Turbidity.
TDS and TSS: Total dissolved solids comprise inorganic salts (principally calcium, magnesium, potassium, sodium, chlorides and sulphates) and some amounts of organic matter that are dissolved in water. TDS of untreated dairy waste water was found to be 2180 ppm and TDS of treated dairy waste water was found to be 1810 ppm. Total Suspended Solids (TSS) refers to small solid particles which remain in suspension form in water as a colloid. It is used as one of the indicator of water quality. It is sometimes abbreviated TSS, but is not to be confused with settle able solids, which contribute to the blocking of sewer pipes. TSS of untreated dairy waste water was found to be 1260 mg/L and that of treated dairy waste water was found to be 40 mg/L.
Salinity and Conductivity: Salinity is the total concentration of all dissolved salts in water. These electrolytes form ionic particles as they dissolve, each with a positive and negative charge. As such, salinity is a strong contributor to conductivity While salinity can be measured by a complete chemical analysis. Salinity of untreated dairy waste water was found to be 1.2 ppm and that of treated dairy waste water was found to be

1.12 ppm. **C**onductivity is a measure of water's capability to pass electrical flow. This ability is directly related to the concentration of ions in the water .These conductive ions come from dissolved salts and inorganic materials such as alkalis, chlorides, sulfides and carbonate compounds. Compounds that dissolve into ions are also known as electrolytes .Conductivity of untreated dairy waste water was found to be 3.15 mS while conductivity of treated dairy waste water was found to be 3.2 mS

BOD and COD: BOD (Biochemical oxygen demand) is the pollution index of any water sample. Among the wastewater parameters, BOD is widely used as a primary indicator to gauge water pollution. BOD provides information about the amount of biodegradable substance present in wastewater. BOD of untreated dairy waste water was found to be 1445 mg/L and that of treated dairy waste water was found to be 13.6 mg/L. The COD test is widely used as a means of measuring the organic strength of effluents. This test allows measurement of waste of in terms of the total quantity of oxygen required for oxidation to CO2 and H2O. During the determination of COD, organic matter is converted to carbon dioxide and water regardless of the biological assimilability. COD of untreated dairy waste water was found to be 4410 mg/L and that of treated dairy waste water was found to be 32 mg/L. BOD and COD of samples was found to be within permissible range. Results of physico-chemical analysis of untreated and treated dairy water are given below (Table 1 and Table 2)

Table - 1: Physico-chemical parameters of untreated dairy waste water. (1ppm = 1000mg/l)

Parameters	**Untreated dairy waste water**
pH	6.4±0.1
TEMP. (^{O}C)	28±2
TURBIDITY (NTU)	25±0.85
TDS(ppm)	2180±50
SALINITY(ppm)	1.2±0.73
CONDUCTIVITY(mS)	3.15±1
BOD (mg/lit.)	1445±30
COD(mg/lit.)	4410±60
TSS (mg/lit.)	1260±60

Table - 2: Physico-chemical parameters of treated dairy waste water(1ppm = 1000mg/l)

Parameters	**Treated dairy waste water**
pH	7.4±0.5
TEMP. (^{O}C)	25±0.5
TURBIDITY (NTU)	20±1
TDS (ppm)	1810±20
SALINITY(ppm)	1.12±0.05
CONDUCTIVITY(mS)	3.26±0.5
BOD (mg/lit.)	13.6±0.8
COD(mg/lit.)	32±4
TSS (mg/lit.)	40±5

b) Biological analysis

Biochemical analysis of dairy waste water shows the presence of protein, carbohydrate, fat and lactose(Table 3 and 4).

Table 3: Biological parameters of untreated dairy waste water (in %)

Parameter	**Amount in percentage (%)**
Total fat	0.0185
Protein	0.83
Carbohydrate	0.562
Lactose	0.57

Table 4: Biological parameters of treated dairy waste water(in %)

Parameter	**Amount in percentage (%)**
Total fat	0.0036
Protein	0.131
Carbohydrate	0.0064
Lactose	Absent

c) Pathogen detection

The untreated dairy waste was analysed for presence of any type of pathogen. No pathogen was detected in the sample (Table 5).

Table 5 : Analysis of pathogens in untreated dairy waste water

Microorganism	Media	Growth characteristics	Actual Growth result	Results
Saccharomyces sp.	Yeast peptone dextrose agar	White to light yellow coloured colonies	Yellow coloured colonies appeared	+
Bacillus subtilis	Bacillus cereus selective Agar	Colonies with a distinctive turquoise blue colour appears.	Colonies with a distinctive turquoise blue colour appeared	+
Lactobacillus	Lactobacillus selective Agar base	Colonies can range from yellow, orange to rust, or brick in colour	Yellow coloured colonies appeared	+
Salmonella typhimurium	Brilliant green agar	Red to pink-white colonies surrounded by a red zone in centre	No Red to pink-white colonies appeared	-
Escherichia coli 0157:H7	Brilliance selective agar	Pink coloured colonies	No pink coloured colonies appeared	-
Listeria monocytogenes	Listeria selective enrichment broth	Brown Grey colonies with black centre	No brown grey colonies appeared	-
Mycobacterium paratuberculosis	Löwenstein-Jensen medium	• Nonpigmented, rough, dry colonies	No colonies appeared	-
Campylobacter jejuni	Campy CVA Agar	Greyish colonies	No greyish coloured colonies appeared	-
Leptospira sp.	Fletcher's Media	Giemsa stain shows tightly coiled spirochaetes	No spirochaetes observed	-
Pseudomonas aeruginosa	Pseudomonas isolation agar	Green coloured colonies	No green coloured colonies appeared	-

d) Production of SCP

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

Table 6: Values of dry cell weight of biomass

Result of biomass yield	Amount
Biomass yield (g/L) Dry cell weight of *Saccharomyces cerevisiae*	0.32
Biomass yield (g/L) Dry cell weight of *Lactobaccillus acidophilus*	0.16

IV. Results And Discussions

Amongst the physico-chemical parameters the pH of untreated waste water was found to be 6.4 while pH of treated dairy waste was found to be 7.4. The pH of dairy waste water was found to be suitable for the production of SCP. Turbidity of untreated dairy waste water was found to be 25NTU and that of treated dairy waste water was found to be 20NTU. There was not much difference between both the dairy waste water samples. Turbidity of untreated dairy waste water was found to be within permissible limits. TDS of untreated waste was found to be 2180mg/L and treated dairy waste was found to be 1810mg/L. Salinity of untreated dairy waste water was found to be1.2 ppm and that of treated dairy waste water was found to be 1.12 ppm. Salinity of both samples was found to be in approximately in same range . BOD of untreated dairy waste water was found to be 1445 mg/L and that of treated dairy waste water was found to be 13.6 mg/L. COD of untreated dairy waste water was found to be 4410 mg/L and that of treated dairy waste water was found to be 32 mg/ L .The BOD and COD levels were high in untreated dairy waste water due to presence of lactose. However the treatment plant was successful in reducing both BOD and COD.Biochemical analysis of untreated and treated dairy waste samples showed the presence of protein, fat and carbohydrates. Lactose was present only in untreated dairy waste water. The percentage of protein was 0.83% which was comparatively more than that of treated dairy waste water. The total amount of protein ,carbohydrates and fats in untreated dairy waste water were found to be suitable according to the requirements for the growth of SCP . Lactose was detected only in the untreated dairy waste water sample. The percentage of lactose was found to be 0.57. Lactose is necessary constituent for the biomass production. Lactose act as substrate for the SCP production.

Pathogen analysis was done to check the presence of any bovine pathogen in untreated dairy waste water sample.Thus,the physico analysis showed that untreated dairy waste water was suitable as substrate for the SCP. The main parameters such as pH,TSS,TDS , BOD , COD etc were found to be in minimal range for the growth of SCP .Lactose being present in the untreated dairy waste water in addition to protein, fat and carbohydrates. So, the untreated dairy waste water was chosen to be as suitable substrate for the SCP production. Although the physico chemical and biological analysis proved the untreated dairy waste water to be a suitable substrate for the SCP production but also it was a essential to analyse for any type of bovine

pathogen present in it.In Pathogen analysis no pathogen was detected . Thus the samples was found to be suitable according to the requirements for the SCP production. Shake fermentation method was used for the biomass production. The biomass after being converted into dry cell weight was calculated. The SCP produced was obtained in dried powder form. .

V. Conclusion

Dairy waste can act as substrate for the growth of culture and production of biomass. This microbial biomass can be further used for production of edible unicellular organisms- SCP. On one hand organic load decreases and on other hand biomass yield increases that will lead to production to food supplement. Untreated dairy waste have constituents such as fat, carbohydrate ,proteins and lactose that enhances culture growth and the dry cell mass obtained in powder form is thus be further used as SCP. Physico-chemical analysis, Biochemical analysis and microbial detection inferred a successful yield of biomass for SCP production. Thus it is concluded that the culture biomass produced during shake flask fermentation process can be used as a rich source of protein supplement. 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. This combined approach will not only educe impact of untreated dairy waste on environment but also prove to be boon in Food industry by growing microbial cultures for production of SCP.

References

[1]. Anikwe, M.A.N. and Nwobodo, K.C.A., (2006) Long term effect of municipal waste disposal on soil properties and productivity of sites used for urban agriculture in Abakaliki, Nigeria. Bioresources Technol. 83:241-251.
[2]. Bozakouk,A. H. (2002) Acid hydrolysis of Phragmites austral; is powder for production of single cell protein by *Candida utilis*. Journal of Research, 98:876-897.
[3]. Chaiudhari, D.H and Dhoble, R.M (2010) Performance evaluation of effuluent treatment plant of dairy industry. Current World Enviornment ,5(2):373-378.
[4]. Dairytech India 2011.
[5]. Moeine, H., Nahvi,I. andTavassoli, M. (2004) Improvement of production and BOD removal of whey with mixed yeast culture. Electronic Journal of Biotechnology – Chile. ISSN: 0717- 3458. 7:3,
[6]. Najafpur, Ghaswm D., (2007) Single Cell Protein .Biotechnology advances. Biochemical Engineering and Biotechnology Adavances, 332-341.
[7]. Nasseri, A.T., Rasoul-Amini, S., Moromvat, M.H. and Ghasemi, Y.(2011) Production of Single Cell Protein from Fruits waste. American Journal of Food Technology, 6(2): 103-116.
[8]. Nigam, N.M., (2000) Cultivation of *Candida langeroniii*n sugarcane bagasse hemicellulose hydrolysate for the production ofsingle cell protein. World Journal of Microbiology and Biotechnology, 16,367-372.
[9]. Schultz, N., Chang, L. Hauck, A.,Reuss,M. and Syldatk. (2005) Microbial production of single-cell protein from deproteinized whey concentrates. Appl. Biochem. Biotechnol.
[10]. Zubi,W. (2005) Production of single cell protein from base hydrolyzed of date extract byproduct by the fungus *Fusariumgraminearum*MSc. Thesis, Benghazi: Garyounis University, 19:167-225.

IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

Gour Suman. “Characterization of Dairy Waste and its Utilisation as Substrate for Production of Single Cell Protein.” IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) , vol. 3, no. 4, 2017, pp. 73–78.