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Biodegradation of Lipid Rich Dairy Effluent by Bacterial Consortium

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Abstract:*In thepresent study biodegradation of lipid rich dairy effluent by a consortium of lipase producing bacteria was carried out. Lipase producing bacteria were isolated from the soil by serial dilution plate technique and spotting individual colonies onto tributyrin agar plates. The bacteria which formed a halo zone were identified as Bacillus coagulans, Bacillus simplex and Trichococcus sp. When the dairy effluent was treated with the three bacterial strains individually and as a consortium, there was a reduction in biological oxygen demand and total lipids. The reduction in the level of total lipids is due to the secretion of lipase by these organisms. The degradation was higher and faster when the effluent water was treated with the consortium than when treated with the organisms individually.*

Keywords:*Biological oxygen demand, lipase, total lipids, dairy effluent, consortium.*

I. Introduction

Due to the increase of population and rapid industrialization, the problems of sewage disposal and industrial waste water management haveassumed enormous proportions requiring urgent attention. Dairy industry is one among the polluting food industries because of its large water consumption. Due to the increased demand of milk and milk products the dairy industry in India is expected to grow rapidly and thereby the dairy effluent is expected to pose environmental pollution problems in the near future.

For the degradation of organic matter, various industries have replaced the use of chemicals which are detrimental to the environment and equipment, by new processes that use enzymes under less corrosive conditions [1]. The application of lipase producing microorganisms to degrade oil and fat has become an interesting strategy because it eliminates the need for pretreatment processes and the lipid residues are converted into carbondioxide and water, and cost of treatment is less[2, 3]. Hence persistent screening for new microorganisms that secrete lipolytic enzymes will open new ways to solve many environmental problems.

In the present study, biodegradation of lipid rich dairy effluent using lipase producing bacteria – Bacillus coagulans, Bacillus simplex and Trichococcus sp., and a consortium comprising these three organisms has been investigated.

II. Materials and methods

Isolation of bacteria:Lipase producing bacteria were isolated from the soil in the premises of a dairy by serial dilution and plate technique and by spotting individual colonies onto tributyrin agar plates. Colonies which formed a halo zone on tributyrin agar plates were selected for further studies. The bacteria were identified by staining, morphological and biochemical characters[4]. The isolated organisms were stored in nutrient agar slants at 4°C.

Treatment of dairy effluent:Effluent water from a dairy was collected to sterile containersand immediately brought to the laboratory. The effluent water (1 L)was transferred to five conical flasks of 2L capacity. 10% v/vof overnight cultures of the Bacilluscoagulans, Bacillussimplex, Trichococcusspin peptone water were added to the effluent water taken in separate conical flasks.10%v/vconsortium comprising Bacillus coagulans, Bacillus simplex, Trichococcusspwas also added to another conical flask containing 1L of effluent water.All the flasks were incubated at 30^0C at 200 rpm. Samples were taken at regular intervals of 48h from each flask andbiological oxygen demand(BOD), total lipids, activity of lipase enzyme and pH were determined.

Methods**:**Estimation of protein, reducing sugars and total lipids were done by the methods described by Lowry et al[5], Miller et al[6], Christine and Stephanie[7], respectively. Total solids were determined by evaporating a known volume of the effluent in a previously weighed petridish and noting down the weight after evaporation. The pH of the effluent water was checked in a pH meter ELICO Model L1 120. BOD was determined by Winkler's Iodometricmethod[8]. Activity of lipase enzyme was determined by the method of Safarnick[9], by estimating the amount of free fatty acids liberated. Activity of the lipase enzyme is defined as the µ moles of oleic acid liberated /minute/ml of the culture supernatant.

III. Results and Discussion

In the present study lipase producing bacteria were isolated from the soil and they were identified as Bacillus coagulans, Bacillus simplex and Trichococcussp, by staining, morphological and biochemical characters **(TABLE1).**The degradation of lipid-rich dairy effluent usingthe three isolated bacteria individually and as a consortiumwas investigated. The dairy effluent contained total solids 3600 g/L, protein 304 mg/L, reducing sugar 472.5 mg /L, BOD 1600 mg/ L, lipid content 2600g/L, pH 4.4, before the treatment **(TABLE 2).**

When the dairy effluent was treated with the bacteria individually and as a consortium, there was a reduction inBOD. BODis the amount of dissolved oxygen needed by aerobic organisms present in a body of water to breakdown theorganic materials at certain temperature over a specific time period [10]. The BOD valueof waste water reduced from1600 mg/L to 400mg/L by the three bacterial strains when treated individually. The decrease in BOD was faster when the consortiumwas used than theindividual organisms.There was a considerable decrease in BOD on the third day itself, when consortium was used for the treatment. The BOD reduced to 200mg/L at the end of the treatment**(Fig 1)**.

The lipid content was 2600g/L before treatment, which reduced to 900g/L,1200g/L and 400g/L by Bacilluscoagulans,Bacillus simplex and Trichococcus sp. respectively. When consortium was added the lipids reduced to 300g/L**(Fig 2)**. The secretion of lipase enzyme was highest on the sixth day for Bacillus coagulans, Trichococcussp.and for the consortium. For Bacillus simplex the secretion of lipase was highest on the third day**(Fig 3)**. There was a positivecorrelation between the lipase secretion and the reduction in lipid content. The lipase production and decrease in the level of total lipids was higher when the consortium was used for the treatment.

Our results are consistent to those reported by Dharmsthiti and Kuhasuntisook[11]. Prasad and Manjunath[12]have reported the use of B.subtilis, B.licheniformis, B.amyloliquefaciens, S.marsescens, P.aeruginosaand S.aureusin waste water treatment. The average BOD value was reduced from 3200 mg/L to less than 40 mg/ L and lipid content was reduced from 25,000 mg/L to 80 mg/L, respectively within 12 days of incubation. P.aeruginosashowed reduction of BOD value from the day one in palm oil refinery effluent, dairy effluent and domestic water effluent[12]. Single culture of Pseudomonas sp.was highly efficient and showed 83.46% reduction of fat and 95.81% reduction of COD[13]. The degradation of lipid rich waste water is reported by Mongkolthanaruk and Dharmishtialso[14].

In our study thereduction in the level of lipids may be due to the secretion of lipase enzyme by Bacillus coagulans, Bacillus simplex and Trichococcus sp. The decrease in the lipid content may have contributed to the reduction in the BOD.Pratuangdejkulet al.,[15]reported that the three strains of lipase producing bacteria such asAcinetobactercalcoaceticusLP_{602},Bacillus sp_{304} and Acinetobactercalcoaceticuscould be successfully used for treatment of lipid rich waste water.

Galactionet al [16] reported that variations in pH may affect the degradation of lipids by microoganisms. So the variationsin pH were monitored during the course of the study. Initial pH of the sample was 4.4. There was a slight decrease in the pH till the sixthday**(Fig 4)**. Thisdecrease may be due to the increase in concentration of free fatty acids formed by the hydrolysis of triglycerides present in the effluent, to fatty acids. The release of the H^+ions by the cells to the reaction medium also contributes to the reduction of pH [17]. The release is carried out during the antiport of the organic compounds of the culture medium towards the cell [17]. After the sixthday pH gradually increases. The increase could be due to the degradation of fatty acids and organic acids having COO^- and OHgroups. Moreover the microbial activity involves degradation ofprotein and releases NH_4^+ions which causes the increase of pH [17]. In our study the small variations in pH have not influenced the degradation of lipids.

Table 1: Staining, Morphological and Biochemical characters of the isolates

MORPHOLOGICAL CHARACTERS	**Bacillus coagulans**	**Trichococcussp.**	**Bacillus simplex**
Colony characters	Small , white, round, convex, smooth, opaque colonies.	Large , greyish white, smooth, mucoid, opaque colonies	Large , dry, entire margin , greyish white , opaque colonies.
Grams reaction	+	+	+
Motility	+	_	+
Shape of the cell	Rod	Cocci	Rod
Biochemical characters			
VP test	+	_	+
Acid from glucose and sucrose	++	+ and -	++
Acid from lactose and maltose	_ _	Only acid production in maltose	_ _
Citrate utilization	_	+	_
Indole production	_	_	_
Catalase	+	+	+
Oxidase	_	_	+ /-
Urease	_	_	_
Methyl red test	+	+	+
TSI	Alkaline slant with alkaline butt	Alkaline slant with acid butt	Alkaline slant with acid butt
Growth between 24hrto 48hr	+	+	_
Growth between48hrsto 72hrs	+	+	+

Table: 2 Characteristics of the dairy waste water before treatment

Parameters	**Values**
pH	4.4
Total solids	3600g/L
Total lipids	2600g/L
BOD	1600 mg/L
Total protein	304mg/L
Reducing sugars	472.5 mg/L

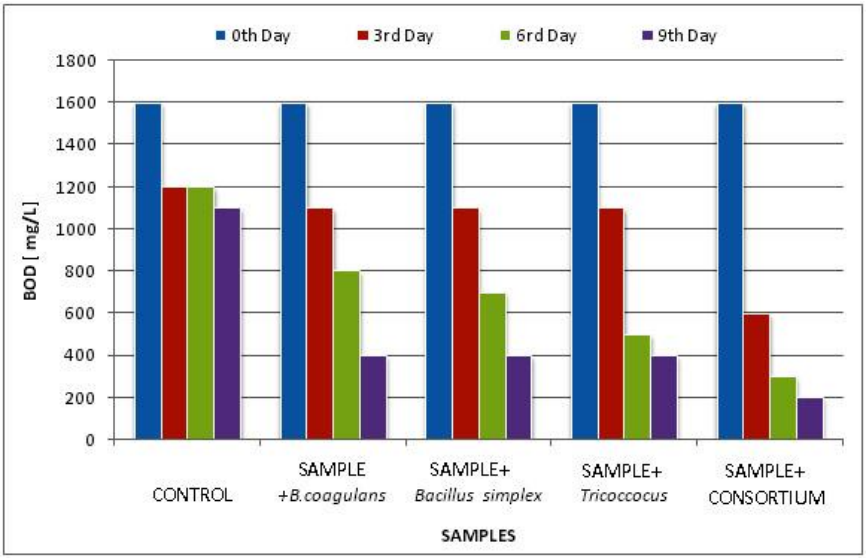

Figure 1: Biological oxygen demand of the effluent water.

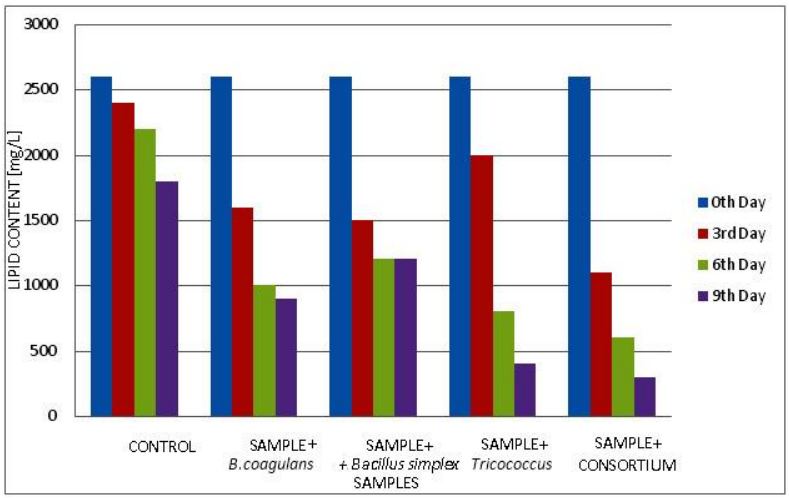

Figure: 2 Amount of lipids present in the effluent water.

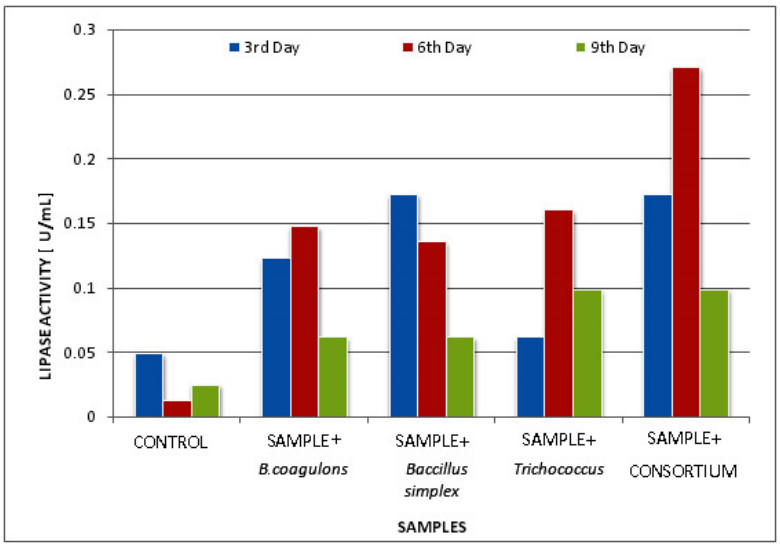

Figure: 3 Activity of lipase enzyme. Enzyme activity is expressed as micromoles of free fatty acids liberated /minute/mL of culture supernatant.

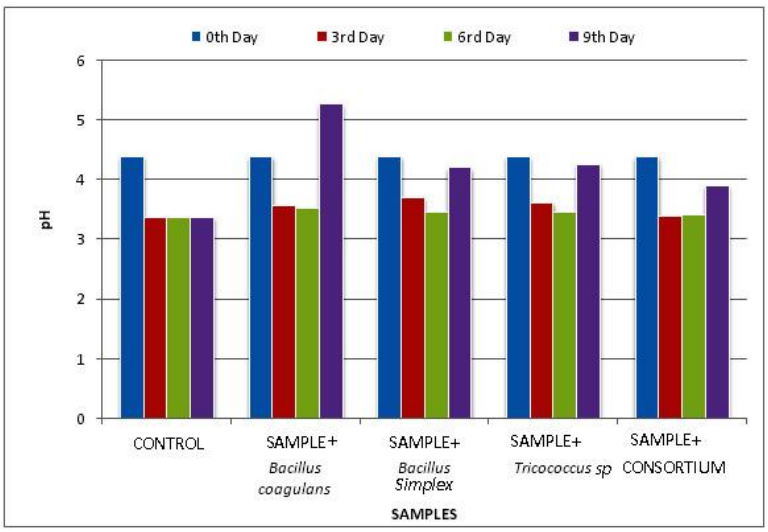

Figure:4.pH of the effluent water.

IV. Conclusions

It can be concluded from our study that the consortium is more efficient in the degradation of lipids in the waste water compared to the individual organisms. There was reduction in BOD from 1600mg/L to 200mg/L, lipids from 2600g/L to 300g/Lwithin nine days of the treatment. This reduction is due to the secretion of lipase by the organisms. Further reduction in BOD values canbe achieved by increasing the inoculum size. However further investigations are required to understand the suitability of using these organisms in the treatment of lipid rich waste water on a large scale.

Acknowledgements

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