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RESEARCH ARTICLE

Experimental studies on lactose biodegradation of dairy effluent by *K.lactis.*

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Abstract

It is inevitable to treat the dairy waste effluents since they are the most polluting ones due to its huge volumes discharged to the inland surface water streams.The COD range of the dairy effluents is about 2000-3000 mg/L. About ten organisms of different species have been tried for finding out their ability to degrade lactose. Among the ten organisms tested three strains were found to be most promising namely *K. lactis, C. laurentii* and *L. plantarum*. The strain *K. lactis*,degraded all the lactose during exponential cell growth phase and removed 67% COD in shake flask cultivation.Based on the above results, a batch and continuous process was performed with *K. lactis* to the optimize and scale up of the biodegradation process.The present studies are carried out to evaluate the performance of various microorganisms in degrading lactose present in dairy effluent and the highly degrading strain of *K.lactis* was identified.

Introduction:-

The dairy industry revolves around the processing of raw milk to different products (e.g. yogurt, cheese, cream, milk, etc)[1] (Rivas et.al20100 .Its processing discharges vast amount of effluents which may result in severe pollution of environment. According to Carvalho et al. the water consumption, depending on production, is between 1-4times that of milk received[2].Dairy wastes, due to their composition, volumes and fluctuating flow rates, represent a problem for municipal wastewater treatment plants therefore, treatment of this kind of effluent seems to be essential [3].Several pretreatment technologies have been developed and applied to treat dairy effluent such as activated sludge system, anaerobic pond, oxidation pond, trickling filter, and combined trickling filter/activated sludge system [4] (Garrido et al., 2001; Perle et al., 1995). Pretreated effluent from these processes have considerable amount of biological and chemical oxygen demand (BOD & COD), oil and grease and also some other pollutants. The pollution load is linked to the wastes organic content which is typically measured as COD and BOD [5].Cheese whey, a dairy industry by-product [6], stands out with a COD significantly greater than the other effluents. In order to eliminate these pollutants, conventional biological treatments of aerobic and anaerobic treatments or facultative digestion are the most commonly used.

Materials& methods:-

Milk industry wastewater:-

The waste used is the permeate from Effluent treatment plant. It was obtained from Vijaya Dairy in Hyderabad. The wastewter was stored in sterile containers in a fridge or a cooling room at 4 ^{o}C and used within a week.

Microbial strains:-

A total of 10 strains were tested for their ability to degrade lactose. Cryptococcus laurentii CY-0301, Cryptococcus flavus CY-0901, Kluyveromyceslactisvar. Lactis ky-0603, Kluyveromycesmarxianusky-0403, Lactococcus Lactissubsp. Cremoris HP and Lactobacillus Plantarumsubsp. plantarumLp 39 were ordered from the Leibniz instituteDSMZ (German collection of microorganisms and cell cultures). In addition, strains of

Lactobacillusplantarum (hereafter referred to as L. plantarumBS), Lactobacillus acidophilus, Saccharomyces cerevisiae and Pichiapastoris were supplied by IRIS.

Media for stock cultures:-
Five different media (broth and agar) were prepared in 2 L(Table.1) Schott bottles. The pH of the media were adjusted by adding NaOH (1M) or HCl (1M). The media was sterilized by autoclaving (121^{o} C, 15 min).

Table 1:- Composition of the media prepared for stock culturing

MRS	**Trypticase soy (TS)**	**Potato dextrose (PD)**	**Universal yeast medium (YM)**	**Yeast extract peptone dextrose(YPD)**
(M.11)[a]	(M.92)[a]	(M.129)[a]	(M.186)[a]	(M.393)[a]
Broth:	Broth:	Broth:	Broth:	Broth:
55.2 g MRS broth media 1 L UPW pH 6.3	17g Bacto™Tryptone 3 g Bacto™Soytone 2.5 g Glucose pH 7.0 5 g NaCl 2.5 g K_2HPO_4 3 g Yeast extract 1 L UPW	20 g Glucose 1 L infusion from potatoes pH 5.6	3 g Yeast extract 3 g Malt extract 5 g Bacto™ peptone pH 6.0 10 g Glucose 1 L UPW	10 g Yeast extract 20 g Bacto™ peptone pH 6.4 20 g Glucose 1 L UPW
Agar:	Agar:	Agar:	Agar:	Agar:
Same as broth with 15 g Agar added	Same as broth with 15 g Agar added	Same as broth with 15 g Agar added	Same as broth with 15 g Agar added	Same as broth with 15 g Agar added

Inoculation of microbial cultures:-
Inoculation with 5% of late exponential phase cultures were done in duplicates. Non-inoculated controls were used.

Cultivation conditions:-
Aerobic organisms were incubated at 25 ^{o}C - 30 ^{o}C with orbital shaking at 150 rpm. Anaerobes were incubated at 30 ^{o}C or 37 ^{o}C without shaking. The growth media changed as the experiments proceeded. To obtain a healthy culture, frozen or freeze dried cultures were first pre-cultured in optimum media as described by the organism's supplier [7]. Freeze-dried cultures of K. marxianus, K. lactis, C. laurentii, C. flavus, L. lactis and L.plantarumLp 39 were re-suspended in their regular media (M.393, M.186, M.186, M.129, M.92 and M.11 respectively) and put to grow on agar plates and in broth. K. marxianus, K. lactis, C. laurentii and C. flavus were inoculated in 250 ml shake flasks containing 100 ml media. L. lactis and L. plantarumLp 39 were inoculated in 20 ml anaerobic tubes containing 17 ml media. The broth cultures were incubated for 24 h, while plates were incubated for 48 h. frozen cultures of L. plantarum BS and L. acidophilus were allowed to thaw before they were pre cultured in anaerobic tubes containing 17 ml media (M.11). The cultures were incubated for 24 h. Agar plates were incubated for 48 h. A fresh culture of S. cerevisiae and a frozen culture of P. pastoris were pre-cultured in closed 50 ml sterile bottles containing 15 ml media (M.393). The cultures were incubated for 24 h. Agar plates were incubated for 48 h.

Cultivation in nutrient solution with added lactose:-
Cultures of *L. plantarum* BS and *L. acidophilus* were inoculated in 25 ml anaerobic tubes containing media as described in Table 3.3.3.a. Cultures of *L. plantarum*Lp 39 and *L. lactis* were inoculated in 25 ml anaerobic tubes containing medium 1 (Table 2).

Table 2:- Media with different concentrations of yeast peptone added for growth of anaerobic microorganisms on lactose.

Medium no.	**Salt premix (ml)**	**Yeast peptone (g/L)**	**Lactose (g/L)**
1	13	40	20
2	14	30	20
3	15	20	20
4	16	10	20
5	17	-	20

Cultures of *K. lactis, C. laurentii, K. marxianus C. flavus, P. pastoris* and *S. cerevisiae* were inoculated into 50 ml

Schott tubes with 10 ml medium containing salt premix, lactose (20 g/L) and yeast peptone (40 g/L).

Microbial growth measurements:-
Microbial growth was monitored by measuring optical density (OD) with Ultra spec 2100 pro spectrophotometer. During all measurements UPW was used as blank and when necessary also as dilutant. The absorbance was at all times measured at 600 λ.
Cell dry weight was determined using a Mettler AE 240 analytical balance, a HeraeussepatechLabofuge 200 centrifuge, centrifuge tubes and a Termaks incubator. Suspended cells (5 ml) were added to two pre-weighed tubes. The tubes and their contents were weighed and centrifuged (2500 rpm, 12 min). The supernatant was discarded, and the cells were washed with ultrapure water prior to a second centrifugation (2500rpm, 12 min). The supernatant was again discarded, and the tubes were left to dry in the incubator at 105^{o}C overnight.

Chemical oxygen demand (COD) measurements:-
Samples for COD measurements were stored in Eppendorf tubes in a fridge at 4^{o}C.The COD content of the samples were determined by using a Spectroquant COD cell test kit from Merck.

pH measurements:-
Measurements of pH were performed with a Radiometer Copenhagen PHM210 Standard pH meter according to manufacturer's instructions. The pH meter was periodically calibrated with buffers of pH 7 and 4.

Results& Discussions:-

Screening of organisms for waste biodegradation: -
For the preliminary screening of ability to degrade lactose, cultivation was performed in small-scale cultures. This enabled the efficient screening of a large number of different organisms at different growth conditions.

Organisms growth characteristics:-
Growth curve experiments were carried out on the three selected strains, K. lactis, C. laurentii and L. plantarumLp 39. This was done to get a better picture of their growth pattern and relationship between growth and lactose degradation. One set of experiments were performed on limited media (media I) and the other on nutrient media (media II and III) (media composition. The results for the cell growth, lactose degradation and pH changes are presented in Figure .1 for K. lactis, Figure 2.forC. laurentii and Figure .3 for L. plantarum. The cell dry-weight obtained and results of COD analysis on nutrient and limited media are presented in Figures 4 and Figure.5 respectively.

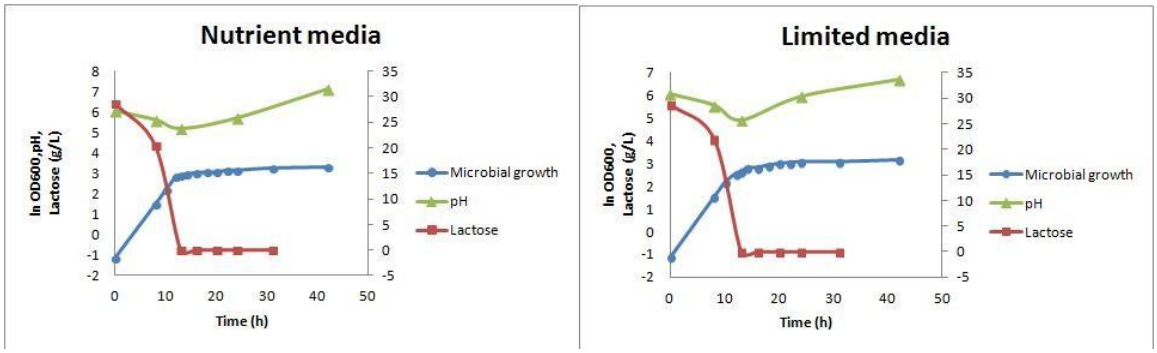

Figure 1:- Lactose degradation and pH changes during *K. lactis* growth (OD) in nutrient media (left side) and limited media (right side)

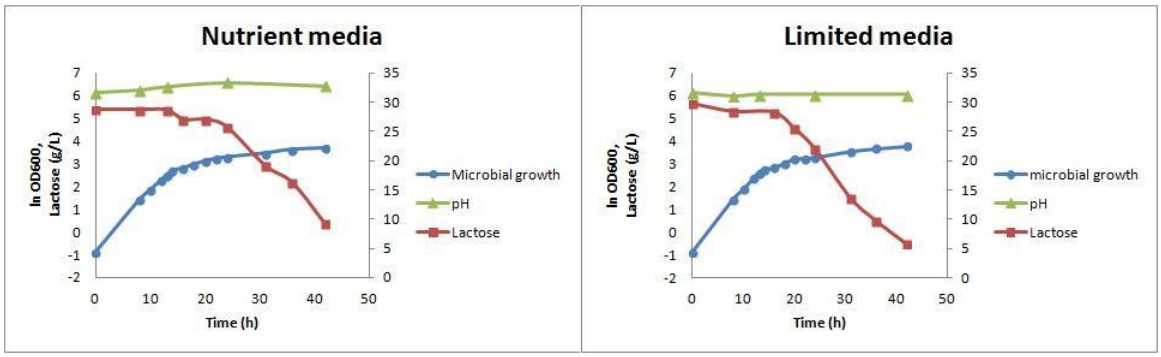

Figure 2:- Lactose degradation and pH changes during *C. laurentii* growth (OD) in nutrient media (left side) and limited media (right side)

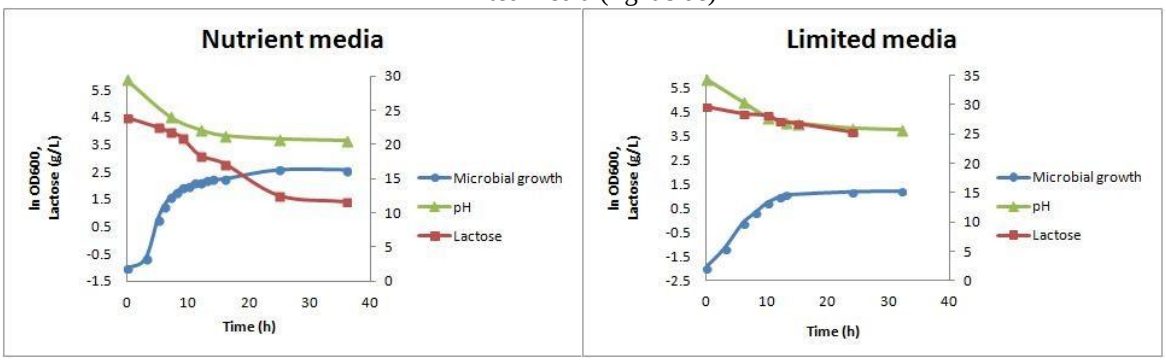

Figure 3:- Lactose degradation and pH changes during *L. plantarum*Lp 39 growth (OD) in nutrient media (left side) and limited media (right side)

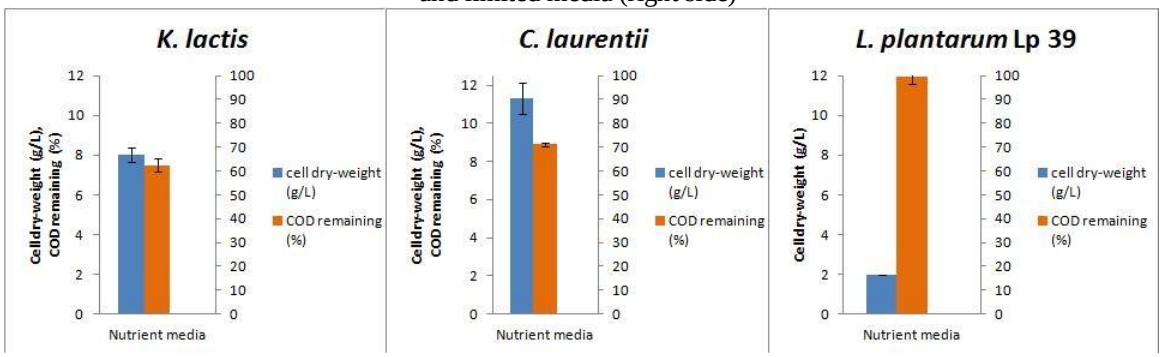

Figure 4:- The Cell dry-weight (g/L) and COD remaining (%) after growth to stationary phase in nutrient media for *K.lactis*, *C. laurentii* and *L. plantarum*Lp 39

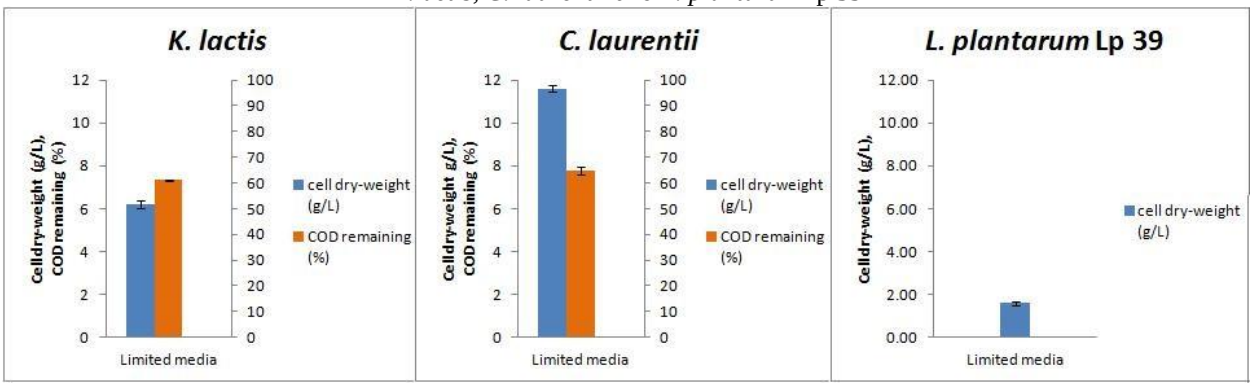

Figure 5:- The Cell dry-weight (g/L) and COD remaining (%) after growth to stationary phase in limited media for *K. lactis*, *C. laurentii* and *L. plantarum*Lp 39. COD were not measured for *L. plantarum*Lp 39

Fermentation:-

After a screening of 10 different organisms, *K. lactis* was chosen based on low nutrient demand, high lactose conversion rate and promising COD removal following batch culture tests. *K. lactis'* ability to grow and reduce the lactose and COD content of dairy waste at the larger scale was tested through fermentation in a 15 L fermenter.

The fermenter was run for 98 h, with two different processes; (1) batch fermentation (0-41 h) and (2) continuously fed fermentation (49-98). Between 41-49 hours addition of yeast peptone was performed to investigate whether nutrients were limiting growth or waste biodegradation.

Conclusion:-

The screening performed in this project gave an indication of which kind of organism will contribute to the overall aim of dairy waste COD removal. *S. cerevisiae* and *P. pastoris*, as expected, did not grow on lactose as a carbon source. The *Lactobacillus* organisms and *L.lactis*proved to be nutrient demanding and their growth resulted in an inefficient COD reduction. *C. flavus* did not reach a high cell density, and did not completely mineralize lactose, resulting in a high concentration of lactose degradation by-products (galactose and/or glucose) in the growth media. Comparatively, *K. marxianus* was relatively inefficient at degrading the lactose. *C. laurentii* degraded lactose largely during cell stationary growth phase, and removed in excess of 30% COD in shake flask cultivation. *K. lactis* degraded all the lactose during exponential growth and removed in excess of 30% COD in shake flask cultivation. During batch fermentation *K. lactis* removed 67% of COD within the first 24 h. Results from trials with different flow rates (200, 300 and 500 ml/h) in continuously fed fermentation, pointed towards a flow rate close to 300 ml /h as optimum for this system.

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