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Physico-chemical characterization of distillery effluent and COD reduction by using *Bacillus badius and Lysinibacillus fusiformis*

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Abstract:

Elimination of pollutants from distillery effluent is becoming increasingly important from environmental and aesthetic point of view. Due to large volume of effluent and presence of certain recalcitrant compounds, the treatment of this stream is rather challenging by conventional method. Therefore, present study characterizes the physico-chemical parameters of distillery effluent. The results of study presents an account of problem such as pH (3.7), colour (black), Electrical Conductivity (15.2 ms/cm), Chemical Oxygen Demand (80500 mg/l) Total solids (22382 mg/l), Suspended Solids (4332 mg/L), Potassium (6080 mg/l), Calcium (1950 mg/l), Chloride (7120 mg/), Sulphate (3250 mg/l), Phosphate (472 mg/l), Sodium (490 mg/l). High concentration of these constituents plus Phosphate, sulphate makes discharge of distillery waste water into water bodies causing problems like eutrophication and other adverse environmental effects. Further studies deals with pure culture of bacteria and role of bacteria in process like COD reduction to develop a better understanding of the phenomenon. This study revealed that the highest COD reduction was obtained with bacterial strain (Bacillus badius) at 30°C after 10 days of incubation period day at pH 8.

Key words: Chemical Oxygen Demand; Spent wash; Bacterial strains; Discolouration

1. Introduction

A rapid change in global economic scenario is due to vast industrialization and incorporation of latest technology in industrial sector. As the fast growing industrialization is a worldwide phenomenon, increase in pollution also moves with same pace. Water is basic requirement in all industrial processes, domestic and commercial activities, so the wastewater generated from different activities contains various contaminants which are harmful for both flora and fauna existing on this planet. Wastewater effluents are intensely coloured and are contaminated with high concentration of suspended solids, dissolved salts and many other recalcitrant compounds. Even small concentration of these compounds present in effluent causes toxicity and foul odors to water. If these effluents are improperly treated, they will pose a serious threat to all aquatic species because hydrolysis of the pollutants in waste water can produce a great deal of toxic products. The natural and the zymogene microorganisms (which come in waters by pollution) constitute a diverse micro biota adapted to different physical and chemical wastewater conditions which are very important for biodegradation (1).

Distillery is one of the most exceedingly polluting and growth oriented industries in India with

reference to the extent of water pollution and the quantity of wastewater generated. Molasses from sugarcane industry is the common raw material used in ethanol production due to its easy availability and low cost (2). India is the second largest producer of ethanol in Asia. At present, there are more than 350 distilleries in India with an installed capacity of approximately 3.5 billion litres of alcohol. Approximately, 50 billion litres of spent wash (waste water effluent) is generated annually in India alone for the production of alcohol. For every one litre of alcohol produced, 10-15 litres of spent wash are generated and thereby a typical distillery producing ethanol from cane molasses generates nearly half million litres of spent wash daily (3-4).

The Central Pollution Control Board (CPCB) categorizes distillery industry among 17 top polluting industries in India (5). The spent wash is highly coloured with an extremely high chemical oxygen demand (COD) load and contains high percentage of dissolved organic and inorganic matter. The biochemical oxygen demand (BOD) and COD, the index of its polluting character, typically range between 35,000-50,000 mg/l and 80,000-1,00,000 mg/l respectively. The dark brown colour of the treated spent wash is mainly due to melanoidin that remain in the effluent undegraded even after conventional treatment.

The present laboratory experiment was designed a) to determine the characteristics distillery effluent. b) to optimize the various parameters for COD removal. c) and to study the removal of COD by bacterial strains at optimized conditions.

2. Materials and Methods

Collection of samples: Spent wash samples were collected in sterilized polythene bottles of 5 L capacity. Temperature and pH of the samples were measured at the time of collection. Immediately after the effluent sampling, the effluent sample was taken to the laboratory and stored at room temperature in the laboratory for further analysis using standard methods.

Physicochemical analysis: All solutions are prepared in pure distilled water with AR grade chemicals. The collected distillery effluent was assessed for various physico-chemical parameters. Physico-chemical properties such as total suspended solids (TSS), total dissolved solids (TDS), total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), chloride, calcium, magnesium, sulphate, nitrogen, phosphorous, sodium and potassium were measured using standard methods. The physico-chemical analysis of distillery effluents is given in Table 1.

Table 1:- Physico-chemical properties of untreated and treated distillery effluents

Parameters	Untreated effluent	Treated effluent
Colour	Black	Dark Brown
pH	3.7	6.9
EC (mS cm^{-1})	15.2	12.1
Suspended solids (mg l^{-1})	4332	292
Total Solids (mg l^{-1})	84642	22382
Total Dissolved Solids (mg l^{-1})	80310	22090
COD (mg l^{-1})	80500	30300
Potassium(mg l^{-1})	6080	2560
Calcium (mg l^{-1})	1950	920
Chloride (mg l^{-1})	7120	3160
Sulphate (mg l^{-1})	3250	1480
Phosphate (mg l^{-1})	472	220
Sodium (mg l^{-1})	490	140

Isolation of micro-organisms

Microbes were isolated by enrichment technique. All the essential requirements were sterilized in the autoclave and necessary glass wares were sterilized in a hot air oven at 250^0C for 2 hrs. One g of moisture free soils were suspended in 100 ml of pre autoclaved effluent basal media in 250 ml Erlenmeyer's flasks in a laminar flow system (Yorko- Horizontal). Nutrient agar and Nutrient broth media were used for isolation of bacteria; a standard composition of both the media was used for the isolation of micro-organisms (6). Media modification was done by dissolving the media constituents in untreated effluent as a substitute of distilled water, all other conditions were kept same as per the standard media preparation technique. Same procedure was followed for isolation of bacterial strains from effluent samples. 1 ml of effluent sample collected from lagoons in place of soil samples, were added in 100 ml of effluent basal media of same concentrations.

These flasks were shaken in an incubator shaker at 150 rpm at 30^0C for 24 hrs. Standard spread plate technique was performed for isolation of bacterial strains. Subsequently, 1.0 ml of suspension from each flask was appropriately diluted by serial dilution technique up to 10^{-7} dilution. Finally 1 ml of suspensions from each dilution was plated on agar medium. These inoculated agar plates were statically incubated in an incubator at 30^0C (BOD-Incubator, NSW) for 2-3 days. Repeated streaking of each single colony on agar media was performed till pure colonies of each culture were achieved. Pure isolates were maintained by regular transfer on agar slants in a refrigerator at 4^0C until used further for screening. All examinations were executed in triplicates.

Screening of the microbial isolates for COD removal of industry effluent

Screening of isolates to check their COD reduction capabilities were done using agar plate method. Nutrient agar media were used for bacteria. Streaking was done incredibly vigilantly, as a result single colony of each pure isolate obtained on the every agar plate. Agar plates were statically incubated at 30^0C for the period of 7 days in an incubator. Each pure isolate was inoculated on the three agar plates. All measurements were repeated three times.

Identification of microbes

The selected isolates were identified from Xcelris laboratory, Ahmedabad and sequencing was get done by National Center for Biotechnology Information (NCBI).

3. Results and Discussion

Effect of Carbon and Nitrogen sources on COD reduction

Mineral salt medium was used along with 200 mg l^{-1} yeast extract and 100 mg l^{-1} for studying effect of various carbon and nitrogen sources of the process of COD reduction. Carbon sources like lactose, glucose, fructose, sucrose and mannitol were sterilized (10 psi for 10 min) separately and added in the reaction mixture with final concentration of 1%. The medium was inoculated with 2% v/v 18 hrs old cultures containing approximately 1.3 x 10^{10} cells ml^{-1}. In case of nitrogen sources, $(NH_4)_2SO_4$ was replaced by peptone, yeast extract, ammonium chloride, urea, with 1% concentration in final reaction mixture. Experiments were carried out in flask containing 100 ml medium and incubated in static conditions at 35^oC.

An attempt was made to test COD reduction of textile effluent by *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2) under influence of various carbon and nitrogen sources. Effect of different salt concentrations on the growth of the organisms and COD reduction was studied by using different concentrations of carbon source in complete medium broth. The change in colour of the medium and growth of bacterial biomass was observed, respectively at different time interval. Study of such effect has been also reported earlier (7-9). Most of the organisms prefer glucose as a source of carbon. Various concentrations of carbon sources like lactose, glucose, fructose, sucrose and mannitol 0.5% were tested for its effect on the process of COD reduction by *Bacillus badius* and *Lysinibacillus fusiformis* under different optimization conditions.

The importance of carbon source to achieve successful COD reduction could be inferred from Figure 1. The micro-organism was able to grow on all tested carbon sources but its COD reduction activity was observed to be influenced by the type of Carbon sources. COD reduction activity at different time intervals i.e 2, 4, 6, 8 and 10 days under static condition was in the range of 50% in

both strains, for carbon source. The potential of *Bacillus badius* and *Lysinibacillus fusiformis* to COD reduction with various carbon source and nitrogen sources is depicted in Figure 1 and 2. Organism growing on these media 44.2 % COD reduction by sucrose as the best carbon source in the 8 days incubation by *Bacillus badius* (strain 1) while in case of *Lysinibacillus fusiformis* (strain 2) 37 % reduction of COD in 10 day incubation period by fructose. However in case of all nitrogen sources, ammonium chloride was the best nitrogen source that yielded highest, 47.2%, COD reduction at day 10, by *Bacillus badius* (strain 1) while *Lysinibacillus fusiformis* (strain 2) 34.8 % reduction of COD by ammonium chloride was better than other nitrogen sources in case of strain 2.

Effect of temperature on COD reduction (%)

The effect of temperature on COD reduction of untreated and treated textile effluent was studied as shown in Figure 3. It was found that COD reduction rate was excellent after day 10 at all temperature values and become almost constant at 30^{o}C and 35 ^{o}C thereafter , which may be due to loss of enzymatic activities at higher temperature, as proteins are not stable at high temperature (10-11). Maximum reduction of COD 53.0 % by strain 2 at 35^{o}C after 8 day of incubation, while in case of strain 1 it was 58.9 % at 30 ^{o}C, after 10 days of incubation period. These results are in accordance with the studies carried out by (12). Therefore, 10 day was considered optimal for COD maximal removal by *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2). The COD reduction prototype of bacteria clearly indicated that the degradation was mildly temperature dependent. Since the microorganism is more sensitive towards the temperature, such reactions are also more sensitive for changes in temperature. Thus temperature of 35 and 30 ^{o}C was considered optimal for COD reduction of textile effluent by the bacterium *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2).

Effect of pH on COD reduction- The effect of pH on COD reduction by potential of *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2) was studied as shown in Figure 4. It was found that there was a linear increase in % of COD reduction of textile effluent with increasing pH, this may attributed to growth of bacterial culture at higher pH values. Maximum COD reduction was observed at pH 8.0 and 9.0 by both the bacterial strains. Effect of pH was more profound on COD reduction with increasing period of incubation shown in Figure 4. Equilibrium COD reduction was favoured by neutral pH range and maximum biodegradation by the bacterium was observed at pH 8.0 and 9.0 by bacteria was also reported by (9). It is clearly evident from Figure 4 that there was more % COD reduction (57.1 %) at pH 8.0 by strain 2 after day 10 of incubation period as compared with strain 1 as reduction of COD 60.4 % at pH 9.0 after day 10 at its maximum level of reduction.

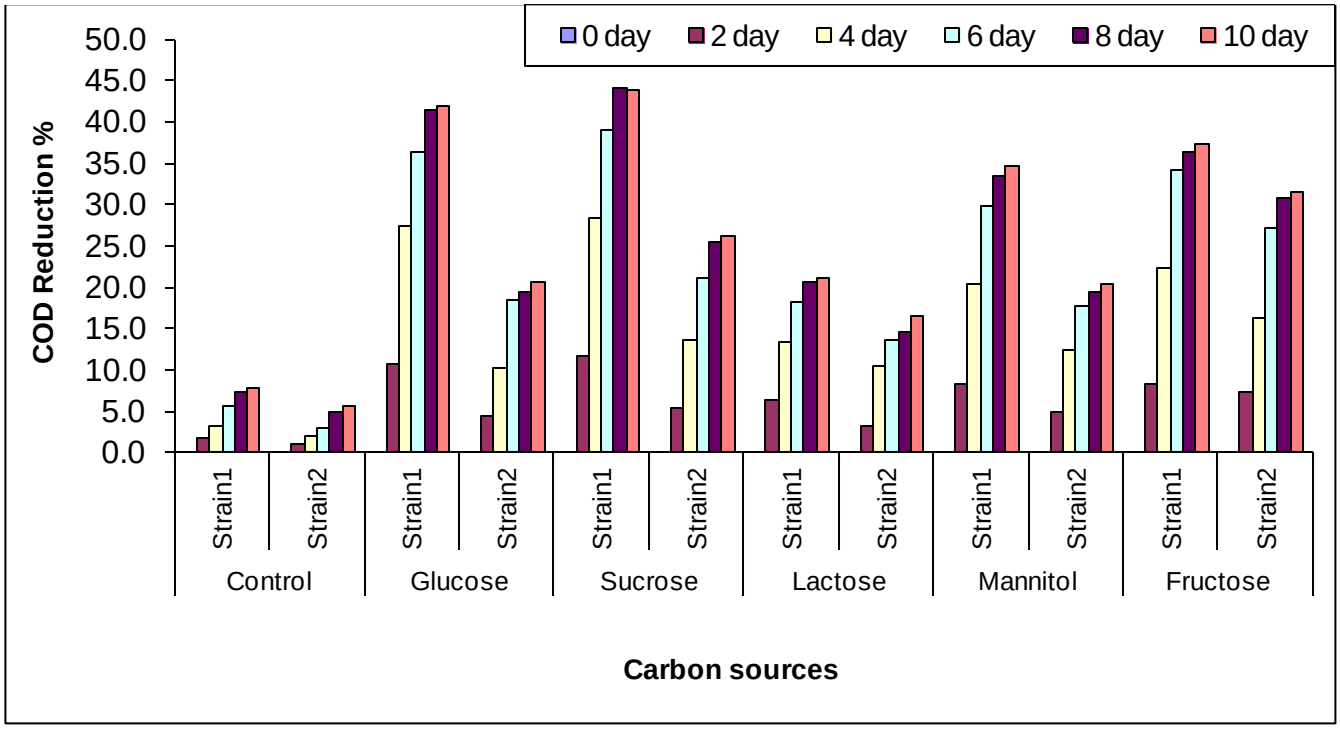

Fig 1: Effect of different carbon source on COD reduction % in distillery effluent in 10 days of incubation period by *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2)

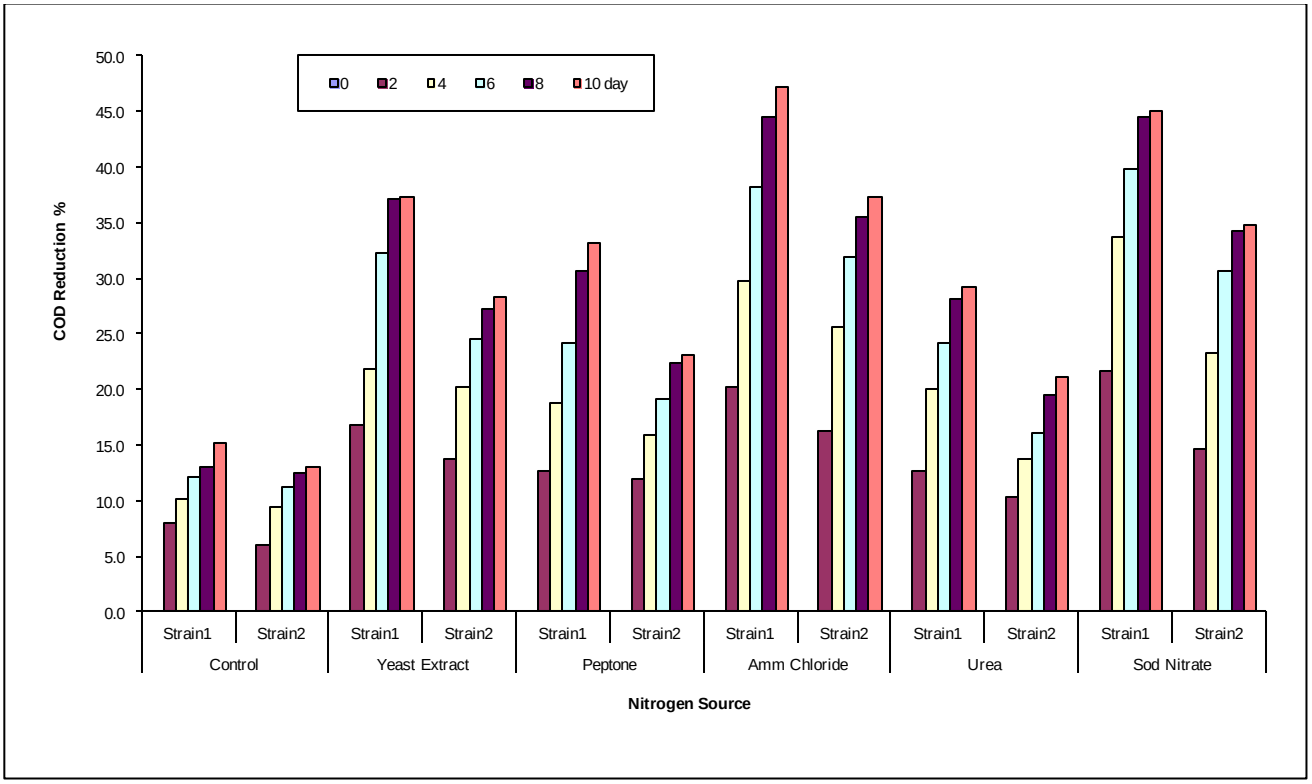

Fig 2: Effect of different Nitrogen sources on COD reduction % in distillery effluent in 10 days of incubation period by *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2)

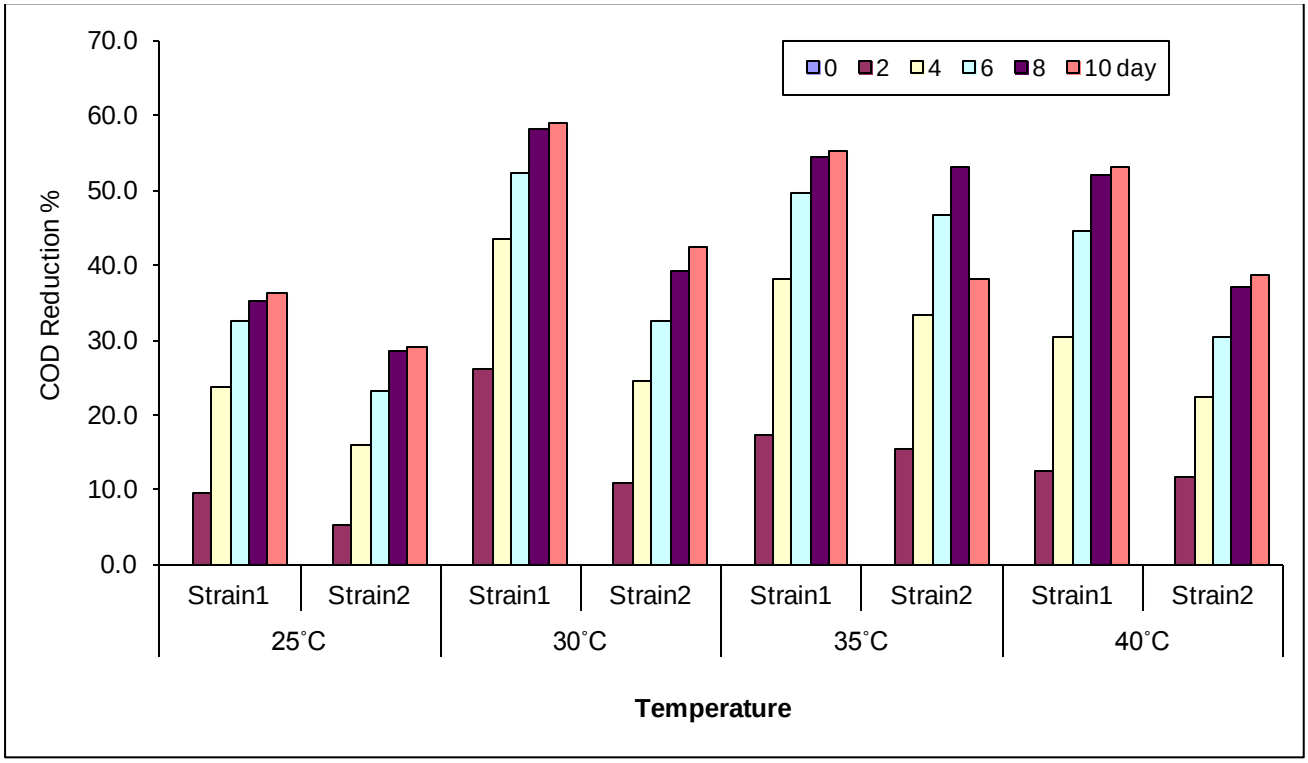

Fig 3: Effect of different temperature on COD reduction % in distillery effluent in 10 days of incubation period by *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2)

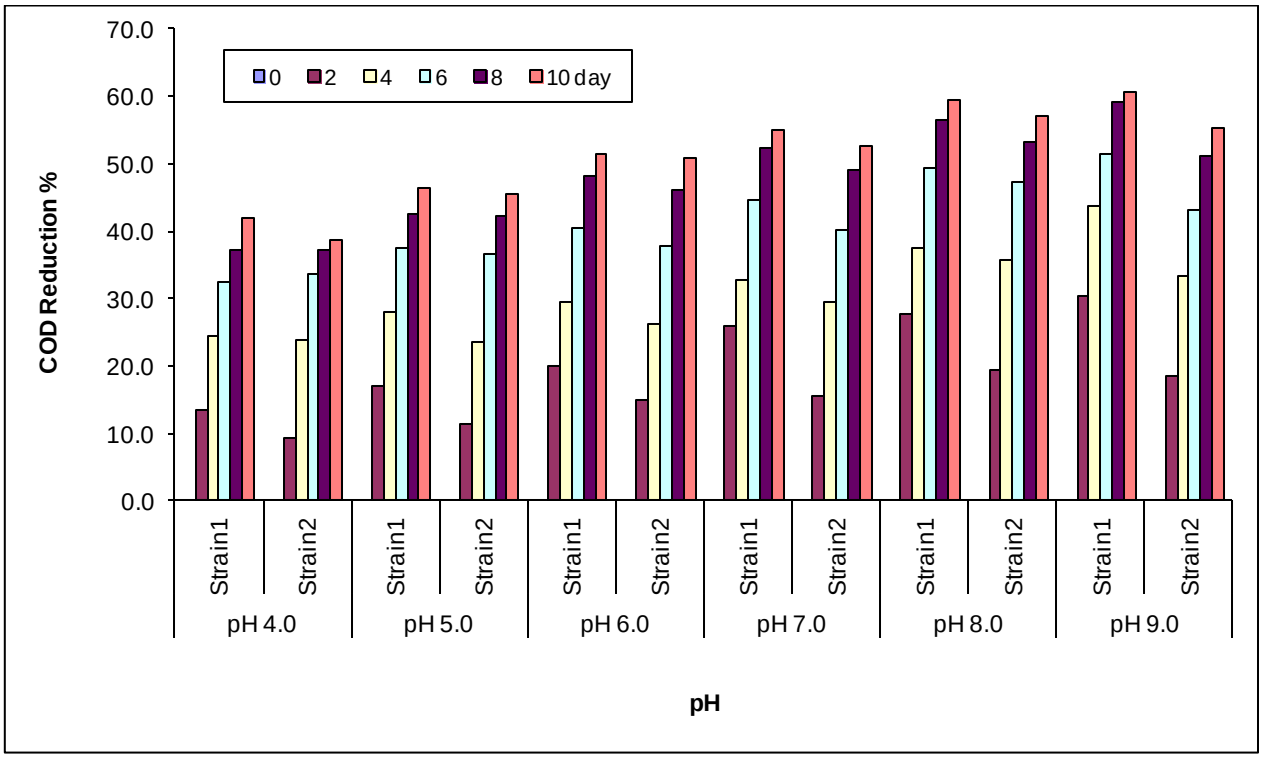

Fig 4: Effect of different pH on COD reduction % in distillery effluent in 10 days of incubation period by *Bacillus badius* (strain-1) *and Lysinibacillus fusiformis* (strain-2)

4. Conclusion

In this study distillery effluent COD was reduced by bacterial strains which was isolated from soil samples of disposal site of distillery industry. The present study provides important baseline information for the use of microorganisms for the reduction of COD from effluent. The study has resulted in the isolation of two very efficient bacterial strains (*Bacillus badius and Lysinibacillus fusiformis*) possessing high carbon-utilizing efficiency, suggesting a potential role for exploitation of these isolates in COD reduction from such effluents. This study revealed that the highest COD reduction was obtained with bacterial strain (*Bacillus badius)* at 30°C after 10 days of incubation period day at pH 8. COD reduction by microbial from distillery stillage therefore shows great promise as a cost-effective, environmentally safe biotechnology for the treatment of industrial wastewater.

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