Int.J.Curr.Microbiol.App.Sci (2020) *9*(5): 1014-1028

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 5 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.905.111

Investigations on Microbial Flora Variation in Water Generated at Various Sugar Production Stages

Seema Paroha* and Shikha Singh

National Sugar Institute, Kanpur, India

**Corresponding author*

Keywords

Bacteria, fungi, sugar industries, effluent

Article Info

Accepted:
10 April 2020
Available Online:
10 May 2020

A B S T R A C T

The water samples of different sugar producing stages from eight selected sugar factories were collected to explore the possibility of its utilization as fresh water. Samples were collected from Fresh / Raw water; 1st body condensate; 2nd body condensate; 3rd body condensate; 4th body condensate; Pan condensate; UGR Cold; UGR Hot; ETP Inlet and ETP Outlet. On an average the macroscopic characters exhibited off white colour, round shape, flat elevation and rough/slimy appearance in all 8 sugar factories whereas microscopic characters of these water samples showed rod /cocci shape and both gram positive and grams negative bacteria in all eight selected sugar factories except vapour condensates exhibited negligible growth of the same. This indicates that vapour condensates are most promising and can be converted into fresh water after required processing. Various Bacterial strains were identified *viz E.coli, B.subtilis, P.putida, Salmonella spp, S.aureus and B.cereus etc.* It was observed that fungal colony was only seen in water sample of fresh water and in ETP inlet and outlet. Maximum numbers of fungal species identified were *Alternaria gaisen, Aspergillus flavus, Aspergillus candidus, Aspergillus niger, Aspergillus nidulans, Peniciilium pinophillum and Trichoderma viride*, this database created a novel record of the fungal diversity associated with sugarcane industrial effluent

Introduction

The most important crop from which sugar can be produced in commercial quantity are sugarcane. India is a largest sugar producing country. Sugar industry has been classified in solid, liquid, and gas Pawar *et al.*, (1998) under seventeenth categories of most polluting industries (Red industry) by the central pollution control board (CPCB) in India CREP-CPCB, (2003). Sugar mills account in the industries which discharge huge amount of effluent per day without any or partially treatment during the crushing season Vinish kathuria, (2014).

Government has certain norms due to control water because it is the main raw material for production process, in each and every stage of manufacturing has lots of complicity on utilizing water in proper manner. The wastewater generated by the sugarcane industries are characterized by their high levels of BOD, COD, pH, TSS and TDS.

Sugar cane industry generates 0.2-1.8 m3/tonne waste water with COD 1800 to 3200 mg/L, BOD 720 –1500 mg/L. Rajeshwarai & Balakrishnan (2000) which are causes for developing water pollution when left untreated. The reason for because sugar industry effluent have high organic matter content such as sugar oils, fats, grease and proteins which have bad impact on the quality of ground water Brown & Skougstad (1970).

Boiler house mainly contributes to the production of air pollution and have little share in water pollution Bevan (1971)This leaves a foul smelling (Hydrogen sulphide) gas which in turn can precipitate iron and any dissolved salts, turning the water black and highly toxic for aquatic life. The dissolved oxygen should be normally at least 5 mg/litre for the survival of fishes and other aquatic life Avasan & Rao (2009).

Discharge of water with high TDS level would have adverse impact on aquatic life, render the receiving water unfit for drinking, reduced crop yield if used irrigation, and exacerbate corrosion in water system and pipe. Sugar factory effluent produces obnoxious odour and unpleasant color when released into the environment without proper treatment. Farmers have been using these effluents for irrigation, found that the growth, yield and soil health were reduced. Along with the effects of various industrial effluents on seed germination, growth and yield of crop plants have captivated the attention of many workers Rahman *et al.,* (2002, Street *et al.,* (2007).

As we know sugar is organic molecule and process of sugar itself contain organic matter which when come in various sections escape in form of vapours or dissolved form in various streams. Thus wastewater of sugar industry contains high levels of organic matter, which serves as food for Bacteria. This results in the increase of these microorganisms in the water. The increased number of bacteria then use up all the dissolved oxygen in the water. On complete exhaustion of oxygen the bacteria begin to break down the chemicals in the water stream to get them oxygen.

The life in effluent is highly diverse and consists of interacting population of microorganisms and effluent fauna, and their activities affect physical, chemical, and biological characteristics of effluent. Microbes are the only entities in the biosphere with an exceptional ability to exploit various organic and inorganic compounds for their growth and transform them to chemical products no longer hazardous to human health and the environment. Some potential fungal and bacterial strains were isolated from sugarcane industrial effluent. Pant and Adholeya (2007b).The aim of this study is to find out pattern of distribution of different microorganisms found in water sample of Sugar Industry collected from different sugar production stages were studied.

Materials and Methods

Collection of water sample

The 8 sugar-mill water samples were collected. Three from the Uttar Pradesh, three from Maharashtra and two from Tamil nadu in sterilized bottles tightly capped. Water samples collected from different sugar production stages were screened for the isolation of potential bacterial and fungal strains. Following samples were collected from the sugar mill-

Fresh / Raw water.
1st body condensate.
2nd body condensate.
3rd body condensate.

4th body condensate
Pan condensate.
UGR Cold.
UGR Hot.
ETP Inlet.
ETP Outlet.

Isolation of bacteria

Samples after being serially diluted in sterile distilled water were plated on nutrient agar plates and then incubated for 48 hrs at 30^{o}C. Discrete bacterial colonies that grew on agar plates were initially grouped on the basis of gram staining and different morphological characteristics such as pigmentation motility and colony forms. Bacterial isolates were then picked, sub-cultured and subjected to further biochemical tests for identification according to Bergey's manual of Determinative Bacteriology.

Physiological and biochemical tests

The physiological and biochemical tests were conducted following the methods of Somasegaran and Hoben (1985) and Josey *et al.,* (1979) respectively, as described by Cappuccino and Sherman (1999) to identify the bacteria. and also confirmed with the help of PIB computer kit. Bryant, (1989].

Isolation of fungi

1ml of the water sample was taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10ml of the water suspension to 90ml of sterile distilled water made different dilution viz., $10^{-1,}$ 10^{-2} and 10^{-3}. 1 ml 10^{-3} dilution was and plated in petridishes containing Potato Dextrose Agar medium (PDA). The pH of the medium was adjusted to 5.6. Streptomycin sulphate (100 ml) was added to the medium to prevent the bacterial growth. The plates were incubated at $25 + 2^{0}$C for five days and fungi appearing on the medium were mounted over a clean slide, stained with lacto phenol cotton blue and observed under the microscope. The fungi were identified by using standard manuals, such as Manual of soil fungi Gillman, (1957). More Dematiaceous Hyphomycetes Ellis, (1976) and Hyphomycetes Subramanian, (1971).

Findings

For the present study, the microbial diversity of water and waste water was carried out. The abundant variation in the types of microorganisms and their distinctive morphological and anatomical features visible to the naked eye enable us to identify the different genera and species and, therefore we can easily assess the extent of their diversity.

Hence, the present study was undertaken to know the bacterial and fungal in different water and waste water samples. For the present investigation samples were collected from 8 sugar factory, three from the Uttar Pradesh, three from Maharashtra and two from Tamilnadu in sterilized bottles tightly capped. Following samples were collected from the sugar mill-Fresh / Raw water;; 1st body condensate; 2nd body condensate; 3rd body condensate; 4th body condensate; Pan condensate; UGR Cold; UGR Hot; ETP Inlet and ETP Outlet;. The bacterial and fungal species were isolated and identified from water samples were recorded.

Visual observation

Samples after being serially diluted in sterile distilled water were plated on nutrient agar plates and then incubated for 48 hrs at 30^{o}C.The results of investigation revealed that the water samples from Pan Condensate, ETP Inlet, ETP outlet, Hot UGR, Cold UGR,

including fresh water samples from all eight selected factories contained microscopic and macroscopic elements, whereas samples of all the vapour condensate exhibited negligible growth of the same. This indicates that vapour condensates are most promising and can be converted into fresh water after required processing. On an average the macroscopic characters of fresh water samples, Pan Condensate water, Hot UGR water Cold UGR water ETP Inlet water and ETP outlet water exhibited off white colour, round shape flat elevation and rough/slimy appearance in all 8 Sugar Factories whereas microscopic characters of these water samples showed rod /cocci shape and both gram positive and grams negative bacteria in all eight selected sugar factories (Table 1-8).

Bacterial flora in the collected samples

Bacteria were isolated from the water samples by serial dilution techniques. Identification of Bacterial Strains was done based on Biochemical Test evaluation. (Table No.9-16).The Biochemical Test investigation of obtained bacterial population revealed that the bacterial strain *viz E.coli, B.subtilis, P.putida, Salmonella spp, S.aureus and B.cereus etc* were present in the samples of fresh water samples, Pan Condensate water, Hot UGR water, Cold UGR water, ETP Inlet water and ETP outlet water in general whereas vapour condensates samples exhibited negligible growth of the same. This indicates that vapour condensates are most promising and can be converted into fresh water after required processing. Ramlake and Bhattacharjee (1992) suggested the polluted habitats found mostly *Pseudomonas* because it is having ability to degrade various pollutants from water samples. Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation Glazer, (1997). Bacteria generally are easier to culture and they grow more quickly than fungi. They are more amenable to molecular genetic manipulations. They are able to metabolize chlorinated and other organic contaminants such as oil and mineralize chemicals using them as carbon or energy source Glazer, (1997). Dahiya *et al.*, (2001a) isolated *Pseudomonas* fluorescens from reactor liquid and found that these bacterial strains are capable of decolourizing melanoidin wastewater up to 76% under non-sterile condition and upto 90% in sterile condition. Decolorization of industrial effluents has been achieved by degradation using bacterial and fungal isolates Suhuttaya, (2009).

According to the result of microbial analysis revealed that the water samples from the Uttar Pradesh factories (I, II, III) Maharashtra sugar mills (IV, V, VI) , Tamil Nadu (VII, VIII) contained bacteria from the family *Enterobactericea* which are facultative anaerobes, coliforms. They produce mixed acid fermentation. As fresh water contains millions of micro flora these microbes are found in abundance. But not all these microbes are pathogenic accept *Salmonella spp*. and *Klebsiella spp*. which may cause some water borne diseases.

Fungal flora in the collected samples

The Fungal evaluation and identification was done in the selected water samples. It was observed that fungal colony was only seen in water sample of fresh water and in ETP inlet and outlet. For identification of fungus culture was mounted on clean slides and stained with lacto phenol cotton blue. The slides were observed under the microscope. The fungus strains were identified based on colony characteristics and staining methods. The mycofloristic composition of effluent sample of different industries varied significantly. Maximum numbers of fungal species identified in all Effluent outlet water samples

were *Alternaria gaisen, Aspergillus flavus, Aspergillus candidus, Aspergillus niger, Aspergillus nidulans, Peniciilium pinophillum and Trichoderma viride*. The genera *Aspergillus* was frequently found in sugar industrial effluent of different sugar factories. Table No. 17. In the course of this study the total 8 fungus species belonging to 7 genera which were capable to degrade the organic matter was observed.

Some potential fungal strains such as *Penicillium pinophilum, Alternaria gaisen, Aspergillus flavus, Fusarium monolifome, A.niger* were also isolated and reported from sugarcane industrial effluent (Pant and Adholeya 2007) A total number of 15 species belonging to 9 genera of fungi were isolated during our investigation in various sugarcane industries of Madhya Pradesh by Awasthi *et al.,* (2011). Diverse fungal cultures have been investigated recently for bioremediation process Aust, (1990) and Bumpus and Aust (1993). By virtue of their aggressive growth, greater biomass production and extensive hyphal reach in the environment, fungi have been seen to perform better than bacteria. The high surface –to – cell ratio of filamentous fungi makes them better degraders under certain niches Ashoka *et al.,* (2002). The fungus have capability to purify the effluent by consumption of organic substances, thus, reducing its COD and BOD, and at the same time to obtain some valuable product, such as fungal biomass for protein rich animal feed or some specific fungal metabolite.

One of the most studied fungus having ability to degrade and decolourize distillery effluent is *Aspergillus* sps. such as *Aspergillus fumigatus* G26, *A. niger, A. niveus, A. fumigates* Ub²60 brought about an average of 69.75% de-colourization along with 70- 90% COD reduction Ohmomo *et al.,* (1987); Miranda *et al.,* (1996); Jimnez *et al.,* (2003), Radhika Agarwal *et al.,* (2010). It is evident from the available data that microbial composition of effluent sample of industry varied significantly. Maximum numbers of fungal species were recorded compare to bacteria

Table.1 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Uttar Pradesh Sugar Factory –I

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Fresh water	Off White	Round	Flat	Rough	Rods	G-ve
2.	1st body condensate	Negligible Growth					
3.	2nd body condensate	Negligible Growth					
4.	3rd body condensate	Negligible Growth					
5.	4th body condensate	Negligible Growth					
6.	Pan Condensate	Off White	Round	Flat	Rough	Rods	G-ve
7.	Hot UGR	Off White	Round	Flat	Slimy	Rods	G+ve
8.	Cold UGR	Off White	Round	Flat	Rough	Rods	G-ve
9	ETP Inlet	Off White	Round	Flat	Rough	Rods	G-ve
10	ETP outlet	Off White	Round	Flat	Slimy	Cocci	G+ve

Table.2 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Uttar Pradesh Sugar Factory –II

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Raw water	Off White	Round	Flat	Rough	Rods	G-ve
2.	1st body condensate	Negligible Growth					
3.	2nd body condensate	Negligible Growth					
4.	3rd body condensate	Negligible Growth					
5.	4th body condensate	Negligible Growth					
6.	Pan condensate	Off White	Round	Flat	Slimy	Rods	G-ve
7.	Hot UGR	Off White	Lawn	Flat	Slimy	Cocci	G-ve
8.	Cold UGR	Off White	Round	Flat	Slimy	Cocci in chains	G+ve
9.	ETP Inlet	Off White	Round	Elevated	Slimy	Cocci in chains	G-ve
10.	ETP Outlet	Off White	Lawn	Flat	Slimy	Cocci	G-ve

Table.3 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Uttar Pradesh Sugar Factory –II

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Fresh water	Off White	Round	Flat	Slimy	Rods in chains	G-ve
2.	1st body condensate	Negligible Growth					
3.	2nd body condensate	Negligible Growth					
4.	3rd body condensate	Negligible Growth					
5.	4th body condensate	Negligible Growth					
6.	Pan Condensate						
7.	Hot UGR	Off White	Lawn	Flat	Rough	Rods in chains	G-ve
8.	Cold UGR	Off White	Lawn	Flat	Smooth	Rods in chains	G-ve/ G+ve
9.	ETP Inlet	Off White	Round	Flat	Slimy	Rods	G-ve
10.	ETP Outlet	Off White	Lawn	Flat	Smooth	Rods in chains	G-ve

Table.4 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Maharashtra Sugar Factory –I

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	DAM raw water	Off White	Lawn	Flat	Slimy	Cocci	G-ve
2.	2nd body condensate	Negligible Growth					
3.	3rd body condensate	Negligible Growth					
4.	4th body condensate	Negligible Growth					
5.	5th body condensate	Negligible Growth					
6.	Pan condensate	Off White	Lawn	Flat	Slimy	Cocci	G-ve
7.	Hot UGR	Off White	Lawn	Flat	Slimy	Cocci	G-ve
8.	Cold UGR	Off White	Lawn	Flat	Slimy	Cocci	G-ve
9.	ETP Inlet	Off White	Lawn	Flat	Rough	Rods	G-ve
10.	ETP Outlet	Off White	Lawn	Flat	Rough	Cocci	G-ve

Table.5 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Maharashtra Sugar Factory –II

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Raw water	Off White	Round	Flat	Rough	rods	G-ve
2.	1st body condensate	Negligible Growth					
3.	2nd body condensate	Negligible Growth					
4.	3rd body condensate	Negligible Growth					
5.	4th body condensate	Negligible Growth					
6.	Pan condensate	Off White	Lawn	Flat	Slimy	Cocci	G-ve
7.	Hot UGR	Off White	Lawn	Flat	Slimy	Rods	G-ve
8.	Cold UGR	Off White	Round	Flat	Rough	Rods	G-ve/ G+ve
9.	ETP Inlet	Off White	Round	Flat	Slimy	rods	G+ve
10.	ETP Outlet	Off White	Round	Flat	Rough	Rods	G+ve

Table.6 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Maharashtra Sugar Factory –III

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Raw water	Off White	Round	Flat	Slimy	Cocci / Rods	G-ve/ G+ve
2.	1st body condensate	Negligible Growth					
3.	2nd body condensate	Negligible Growth					
4.	3rd body condensate	Negligible Growth					
5.	4th body condensate	Negligible Growth					
6.	Pan Condensate	Off White	Round	Flat	Slimy	Rods	G+ve
7.	Hot UGR						
8.	Cold UGR						
9.	ETP Inlet	Off White	Lawn	Elevated	Slimy	Rods	G-ve
10.	ETP Outlet	Off White	Lawn	Elevated	Slimy	Rods	G+ve

Table.7 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Tamil Nadu Sugar Factory –I

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Raw water	Off white	Lawn	Flat	Slimy	Cocci	G-ve
2.	2nd body condensate	Negligible Growth					
3.	3rd body condensate	Negligible Growth					
4.	4th body condensate	Negligible Growth					
5.	5th body condensate	Negligible Growth					
6.	Pan condensate	Off white	Lawn	Flat	Slimy	Cocci	G-ve
7.	Hot UGR	Off white	Lawn	Flat	Slimy	Rods	G+ve
8.	Cold UGR	Off white	Lawn	Flat	Slimy	Rods	G+ve
9.	ETP Inlet	Off white	Lawn	Flat	Rough	Cocci	G+ve
10.	ETP Outlet	Off white	Lawn	Flat	Rough	Cocci	G+ve

Table.8 Macroscopic and Microscopic visual characters of water samples from different sugar production stages of Tamil Nadu Sugar Factory –II

S.No	Sample	Macroscopic Characters				Microscopic	
		Colour	Shape	Elevation	Appearance	Shape	Gram stain
1.	Fresh water	Off white	Round	Flat	Slimy	Rods	G-ve
2.	1st body condensate	Negligible Growth					
3.	2nd body condensate	Negligible Growth					
4.	3rd body condensate	Negligible Growth					
5.	4rd body condensate	Negligible Growth					
6.	Pan Condensate						
7.	Hot UGR	Off white	Lawn	Flat	Rough	Cocci	G+ve
8.	Cold UGR	Off white	Lawn	Flat	Smooth	Rods	G+ve
9.	ETP Inlet	Off white	Round	Flat	Slimy	Rods	G-ve
10	ETP Outlet	Off white	Lawn	Flat	Smooth	Rods in chains	G-ve

Table.9 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Uttar Pradesh Sugar Factory –I

SAMPLE	IMViC				Sugar Fermentation			STARCH HYDROLYSIS HYDROLYSIS	GELATIN HYDROLYSIS	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY TEST	OXIDASE TEST	ENDOSPORE FORMATION	OA / FA ANAEROBIC	Bacteria
	I	MR	VP	C	GLU	LAC	SUC										
Fresh water	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
Pan Condensate	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*
Hot UGR	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*
Cold UGR	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*
ETP Inlet	-	-	-	+	+	-	+	+	-	+	-	+	+	-	-	OA	*P.putida*
	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	OA	*Salmonella sp*
ETP outlet	-	-	+	+	+	+	+	-	+	+	+	+	-	-	-	FA	*S.aureus*
	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*

Table.10 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Uttar Pradesh Sugar Factory –II

SAMPLE	IMViC				Sugar Fermentation			STARCH HYDROLYSIS	GELATIN HYDROLYSIS	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY TEST	OXIDASE TEST	ENDOSPORE FORMATION	OA / FA ANAEROBIC	Bacteria
	I	MR	VP	C	GLU	LAC	SUC										
Fresh water	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	OA	*Salmonella spp.*
Pan Condensate	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
Hot UGR inlet	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*
Cold UGR inlet	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
ETP Inlet	-	-	-	+	-	-	-	+	+	+	+	+	-	+	+	OA	*Micrococcus sp*
	-	-	+	+	+	+	+	-	+	+	+	+	-	-	-	FA	*S.aureus*
ETP outlet	-	-	-	+	-	-	-	+	+	+	+	+	-	+	+	OA	*Micrococcus sp*
	-	-	+	+	+	+	+	-	+	+	+	+	-	-	-	FA	*S.aureus*

Table.11 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Uttar Pradesh Sugar Factory –III

SAMPLE STAIN/ GRAM SHAPE	IMViC				Sugar Fermentation			STARCH HYDROLYSIS HYDROLYSIS	GELATIN HYDROLYSIS	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY TEST	OXIDASE TEST	ENDOSPORE FORMATION	OA /FA	Bacteria
	I	MR	VP	C	GLU	LAC	SUC										
Fresh water	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	OA	*Salmonella sp*
Hot UGR	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	OA	*Salmonella sp*
Cold UGR	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*
	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
ETP Inlet	-	-	-	+	+	+	+	+	-	+	+	+	-	-	-	FA	*Klebsiella sp.*
	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
ETP Outlet	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*

Table.12 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Maharashtra Sugar Factory –I

SAMPLE	IMViC				Sugar Fermentation			STARCH HYDROLYSIS	GELATIN TEST	CATALASE TEST	UREASE TEST	NITRATE TEST	OXIDASE TEST	ENDOSPORE FORMATION	OA / FA	Bacteria
	I	MR	VP	C	GLU	LAC	SUC									
Fresh water	-	+	-	+	+	-	+	+	+	+	+	-	-	+	OA	*B.megateriu m*
	-	--	+	+	+	-	+	+	+	+	-	+	-	+	FA	*B.subtilis*
Pan condensate	-	--	+	+	+	-	+	+	+	+	-	+	-	+	FA	*B.subtilis*
Hot UGR	+	+	-	-	+	+	-	-	-	+	-	+	-	-	FA	*E.coli*
Cold UGR	+	+	-	-	+	+	-	-	-	+	-	+	-	-	FA	*E.coli*
ETP Inlet	-	-	-	+	-	-	-	-	+	+	+	+	+	-	OA	*P. aeruginosa*
	+	+	-	-	+	+	-	-	-	+	-	+	-	-	OA	*E.coli*
ETP Outlet	-	+	-	+	+	-	-	+	-	+	-	+	-	-	OA	*Salmonella sp*
	+	+	-	-	+	+	-	-	-	+	-	+	-	-	FA	*E.coli*

Table.13 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Maharashtra Sugar Factory –II

SAMPLE	IMViC				Sugar Fermentation			STARCH TEST HYDROLYSIS	GELATIN TEST	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY TEST	OXIDASE TEST	ENDOSPORE	OA / FA	Bacteria
	I	MR	VP	C	GLU	LAC	SUC										
Raw water	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
Hot UGR	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	OA	*P. aeruginosa*
Cold UGR	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*
ETP Inlet	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*
	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*
ETP Outlet	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*

Table.14 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Maharashtra Sugar Factory –III

SAMPLE	IMViC				Sugar Fermentation			STARCH TEST HYDROLYSIS	GELATIN TEST	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY	OXIDASE TEST	ENDOSPORE FORMATION	OA / FA	Bacteria
	I	MR	VP	C	GLU	LA	SUC										
Raw water	-	-	+	+	+	+	+	-	+	+	+	+	-	-	-	FA	*S.aureus*
	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
Pan Condensate	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*
ETP Inlet	-	-	-	+	-	-	-	-	+	+	+	+	+	+	-	OA	*P. aeruginosa*
	+	+	-	-	+	-	+	-	+	+	+	+	+	-	-	FA	*P.putida*
ETP Outlet	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	FA	*B.subtilis*

Table.15 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Tamil Nadu Sugar Factory –I

SAMPLE	IMViC				Sugar Fermentation			STARCH TEST HYDROLYSIS	GELATIN	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY	OXIDASE TEST	ENDOSPORE FORMATION	OA / FA	Bacteria
	I	MR	VP	C	GLU	LA	SUC										
Fresh water	-	+	-	+	+	+	+	-	-	+	-	+	+	-	-	FA	*Citrobacter spp.*
Hot UGR	-	-	+	+	+	+	+	-	+	+	+	+	-	-	-	FA	*S.aureus*
Cold UGR	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*
ETP Inlet	+	+	-	-	+	-	+	-	+	+	+	+	+	-	-	FA	*P.vulgaris*
	-	+	-	+	+	+	+	-	-	+	-	+	+	-	-	FA	*Citrobacter spp.*
ETP Outlet	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*

Table.16 Biochemical evaluation of bacterial population and identification of bacterial strains from different sugar production stages of Tamil Nadu Sugar Factory –II

SAMPLE	IMViC				Sugar Fermentation			STARCH TEST HYDROLYSIS	GELATIN TEST	CATALASE TEST	UREASE TEST	NITRATE TEST	MOTILITY TEST	OXIDASE TEST	ENDOSPORE FORMATION	OA / FA	Bacteria
	I	MR	VP	C	GLU	LAC	SUC										
Raw water	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
Pan condensate	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	FA	*E.coli*
Hot UGR	-	--	+	+	+	-	+	+	+	+	-	+	+	-	+	OA	*B.subtilis*
Cold UGR	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	FA	*B.cereus*
ETP Inlet	-	-	+	-	+	+	+	-	+	+	+	+	-	-	-	FA	*Leuconostoc*
ETP Outlet	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	OA /FA	*S.saprophyticus*

Table.17 Macroscopic and Microscopic characters of fungal strains

S.No	Macroscopic Characters	Microscopic Characters	Fungi
Fresh Water			
1	-Pale white color, Texture deeply cottony; -White becoming gray-brown on surface, -Very rapid growth.	-Hyphae broad, not or scarcely septate; - Rhizoids and stolons present; - Sporangiophores brown, sporangiophores ovoid, sporangia rather round,.	*Rhizopus*
ETP Inlet and Outlet			
2	Colonies are green , black or grey in color.	-Conidia are club shaped, -Spores are single or form long chains.	*Alternaria gaisen*
3	-Green in color with yellow or white color margin. -Wrinkled growth. -Become black in color after ageing.	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobose. -Pale green in color.	*Aspergillus flavus*
4	-White cottony growth. -Sometime it gives pale yellow color, black and shades of green. -Erect conidiophores.	-Conidia are one celled, smooth or rough walled. -Conidiophores consists of whorl of phialids.	*Aspergillus candidus*
5	-Black in color. -Powdery texture. -Elevated growth.	-Large dark brown conidial heads. -Conidiophores are smooth-walled and hyaline. -Conidia are globose to subglobose , dark brown to black and rough walled.	*Aspergillus niger*
6	-Produced white, yellow, green and brown color colonies. -Cottony in texture. -Slightly elevated.	-Conidial heads are short columnar. -Conidiophores are usually short , brownish and smooth walled. -Conidia are globosend rough walled.	*Aspergillus nidulans*
7	-Cottony growth. -Center wrinkled. -Mycelium white to orange white. -Conidiogenesis moderate, grayish green in color.	-Colorless hyphae. -Thallus highly branched. -Constricted conidiophores.	*Peniciilium pinophillum*
8	-Light green in color and yellowish conidia scattered throughout the plate.	-Conidia are globose. -Phialids are slender. -Branched conidiophores.	*Trichoderma viride.*

Fungal diversity is very important because of their economic importance as well as their Pathogenicity. Soon the basis of reported fungus and their ability of degradation and enzymatic activity of fungal strain they may use in commercial sector.

A large number of fungal diversity associated with sugarcane industrial effluent and this database created a novel record of the fungal diversity associated with sugarcane industrial effluent. Preparation of database provided a base in solving the problems associated with pollution of sugarcane industry and may become a basis for the management of sugarcane industrial effluent.

Due to the present study we can prepare a database which can provide a base in solving the problems associated with pollution of sugarcane industry and may become a basis for the management of sugarcane industrial effluent and recycle the less contaminated streams

The present investigation was carried out to isolate the most frequently occurring and optimally performing microorganisms from Sugar Industry. Wastewater exhibits dynamic characteristics, it is always better to use consortium over single culture Garcha S *et al.,* (2014). Currently work is underway to construct the microbial consortia based on individual efficacy of isolates.

Mechanism of microorganisms in control of environmental pollution is still being explored. However, it is argued that organisms during bioremediation either eatup/ gobble the contaminants especially organic compounds or assimilate heavy metals themselves, thus effectively degrading specific contaminants / harmful compounds and converting them to nontoxic useable by products. Jai Shanker Pillai *et al.,* (2011).

Acknowledgements

We would like to thank the Director NSI, Kanpur for providing all the facilities to carry out this research work.

References

Agarwal R, Sneh Lata, M Guptaeera, Singh P.2010, Removal of melanoidin present in distillery effluent as a major colorant: A Review, Journal of Environmental Biology, 31, pp 521-528.

Ashoka, C., Geetha, M.S., Sullia, S.B. 2002, Bioleaching of composite textile dye effluent using bacterial consortia. Asian, Journal of Microbial Biotechnology and Environmental Science, 4, pp 65 – 68.

Aust, S.D. 1990, Degradation of environmental pollutants, Microbial Ecology., 20, pp 197 – 209.

Avasan M., Rao S. R, 2009 Effect of sugar mill effluent on organic resources of fish, Pollution Research 20 (2) : 167-171.

Awasthi A.K., Pandey.A.K., Rashmi Dubey 2011, Diversity of fungi in effluents of sugar industries of Madhya Pradesh, International Journal of Environmental Sciences.,1(5), pp 834-839.

Bergey's Manual. 1984, "Bergey's Manual of Systematic Bacteriology", Williams & Wilkins, Baltimore: USA.

Bevan, 1971: The disposal of sugar mills effluents in Queensland 40th proceeding of the T.S.S.C.T. Louisinia October November PP 150 H – 1516

Brown E., Skougstad M.W. Fishman M.J., 1970., Methods for collection and analysis of water samples for dissolved minerals and gases", Techniques of water resources investigations of the U.S. Geological Survey, 160.

Bumpus, J.A., Aust, S.D. 1993, Biodegradation of DDT (1, 1, 1trichloro – 2, 2 bis0) (4chlorophenyl) ethane) by

the white – rot fungus phanerochaete chrysosporium", Appl. Environ. Microb, 53, pp 2001 – 2008.

Cappuccino, J.G., Sherman, N. 1999, "Microbiology; A Laboratory Manual", (34d edn.). Rockland Community College, Suffern: New York. Dahiya, J., D. Singh, P. Nigam (2001a), "Decolourisation of molasses wastewater by cells of Pseudomonas fluorescens immobilized on porous cellulose carrier", Biores. Technol., 78, pp 111114.

CPCB 1989, corporate reasonability for Environmental protection (CREP) List of Industries under 'RED' Category Industries identified by Ministry of Environment & Forests, Govt. of India as heavily polluting and covered under Central Action Plan.

Ellis, M.B (1976), "More Dematiaceous Hypomycetes", Commonwealth Mycological Institute Pub., Kew Surrey, England.

Garcha S., Brar S.K., Sharma K., Performance of a laboratory prepared microbial consortium for degradation of dairy waste water in a batch system, J. Sci. Ind. Res.73 (2014) 346–350.

Garcha S., Kaur N., Brar S.K., 2014, Aerobic granulation strategy for the treatment of dairy waste water, Indian J. Dairy Sci. 67 1–5.

Gillman, J.C. 1957, A manual of soil fungi, Revised 2nd edn., Oxford and I.B.H Publishing company (Indian reprint), Calcutta, Bombay, New Delhi – 250.

Glazer, A.N.1997, Microbial Biotechnology, WH Freeman and Company. New York. pp. 54-58.

Jai Shanker Pillai Naveen Danesh, Puttaiah .E.T, Girish K. 2011, Microbial diversity in solid waste molasses of Sugar Industry, Aranthangi, Tamilnadu" International Journal of Environmental Sciences Volume 2, No 2.

Jimenez, A.M., Borja, R., Martin, A. 2003, Aerobicanaerobic biodegradation of beet molasses alcoholic fermentation wastewater, Process Biochemistry, 38, pp 1275-1284.

Josey,D.P.,Beyhon, J.L., Johnson, A.W.B., Beringer, J.E. 1979, "Strain identification in Rhizobium using intrinsic antibiotic resistance", Journal of Applied. Bacteriology., 46, pp 343-350.

Miranda, P.M., Benito, G.G., Cristobal, N.S., Nieto, C.H. 1996, Colour elimination from molasses wastewater by Aspergillus niger, Bio-resource Technology, 57, pp 229-235.

Ohmomo, S. 1988, Screening of anaerobic bacteria with the ability to decolourize molasses melanoidin", Agricultural and biological chemistry, 57, pp 2429-2435.

Ohmomo, S. 1987, Decolourization of molasses wastewater by a thermophilic strain Aspergillus fumigatus G26, Agricultural and biological chemistry, 51, pp 3339-3346.

Pant, D., Adholeya, A.2007b, "Biological approaches for treatment of distillery wastewater: a review", Bioresource Technology, 98, pp 2321-2334.

Rahman, K.S.M., Banat, I.M., Rahman, T.J., Thayumanavan,T., Lakshmanaperumalsamy, P. 2002, "Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant", Bioresource Technology., 81, pp 2532.

Rajeshwarai K.V., Balakrishnan M., Kansal A., Kusumlata, Kishore V.V.N., 1999, State-of-the-art of anaerobic digestion technology for industrial wastewater treatment, Renewable and sustainable Energy Reviews 4 (2000) 135-156.

Ramlake, P.W., Bhattacharjee, J.W. 1992, Bacterial pollution of drinking water

sources in north Tripura district, Proc Acad Environ Bio, 1(1), pp 1926.

Satyawali, Y., Balakrishnan, M. 2007, "Removal of color from biomethanated distillery spent wash by treatment with activated carbons", Bio-resource Technology, 98, pp 2629- 2635.

Somasegaran and Hoben. 1985, In : Methods in Legume – Rhizobium Technology, University of Hawaii, NIFTAL, Project and Micren, Dept. of Agronomy and Soil., pp 1300.

Street, R.A., Kulkarni, M.G., Strik, W.A., Southway, C., Van Staden, J. 2007, Toxicity of metal elements on germination and seedling growth of widely used medicinal plants belonging to Hyacinthaceae, Bulletin of Environmental Contamination and Toxicology, 79, pp 371-376.

Subramaniam, A., Brown, C.W., Hood, R.R., Carpentor, E.J., Capone, D.G. 2002, Detecting Trichodesmium blooms in Sea WiFS imagery, Deep Sea Res., 49, pp 107-121.

Suhuttaya Jiranuntipon. 2009, Decolorization of molasses wastewater from distilleries using bacterial consortium', Dissertation, DAMRONGLERD, Dr.Ing., pp 183 209.

Vinish kathuria, 2014, Small Industry in India: Are CETPs an Appropriate Response for Controlling Pollution? (PP.245-260).

How to cite this article:

Seema Paroha and Shikha Singh. 2020. Investigations on Microbial Flora Variation in Water Generated at Various Sugar Production Stages. *Int.J.Curr.Microbiol.App.Sci.* 9(05): 1014-1028. doi: https://doi.org/10.20546/ijcmas.2020.905.111