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Physiochemical and Microbial Analysis of Wastewater from Food Industry Effluent near Kuthrel area, Durg District, Chhattisgarh

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Abstract: ***Physiochemical analysis of waste water effluent of food industry has been an intriguing subject in recent years because of issues related to water pollution. Industrial effluent discharge act as one of the major factor that contributes water pollution. Since literatures mentioning physiochemical profile of food industries effluent from Chhattisgarh origin were few. The present study intends to study physico-chemical parameters and microbial analysis of waste water of food industry effluent near kuthrel area from durg district. Results revealed slightly basic nature of the effluent. Value of Dissolved Oxygen (DO) was low whereas, high TDS and COD values were observed suggesting high amount of organic matter present in the effluent. BOD level was under the standard discharge range according to Central Pollution Control Board (CPCB). However microbial analysis of effluent indicated high microbial population with gram negative bacteria and some fungal species which suggests that the effluent must need more adequate and proper treatment before its discharge to reduce microbial load so as to prevent contamination of water bodies into which the effluent might get discharged.***
Keywords: Effluent, food industry, physiochemical parameters, microbial analysis, rural area

I. INTRODUCTION

Food Industries have large influence on Indian economy. Beverage and food processing industries in India is a sunrise sector that has gained prominence in recent years (Srivastava.A *etal*, 2016). However, effluents from various food processing industries are the major cause of water pollution which creates adverse impact on both aquatic and terrestrial ecosystem as well as also alter the physiochemical characteristics of water bodies into which they get discharged. In India, it is found that one third of total water pollution comes in the form of industrial effluent discharge solid wastes and other hazardous waste (Surti H, 2016). Regional pollution control authorities are applying more pressure on industries to reduce their BOD, COD and solids loading to the sewers (Dhanasekar.K *et al*, 2021). Being one of the essential renewable resources, treatment of water is necessary as much as its conservation. Better understanding of physiochemical parameters might also help to spread awareness among the locals about the importance of treatment of wastewater. Food industries in Chhattisgarh state had also been developed in recent years, which is why it is important to analyze the physio-chemical parameters and microbial growth to understand the nature of effluent whether it is polluted or how much it can affect aquatic ecosystem if it is released without an adequate treatment.Since literatures mentioning physiochemical profile of food industries effluent from Chhattisgarh origin were few. The present study intends to study physico-chemical parameters and microbial analysis of waste water of food industry effluent near kuthrel area from durg district.

II. MATERIALS AND METHODS

A. Sample Collection

Samples of waste water effluent from food industry situated near rural areas of kuthrel in durg district were taken in sterile plastic bottles carefully.

B. Physiochemical Analysis

1) pH and Temperature

pH and temperature of the sample were recorded immediately during sample collection by using a pH meter and thermometer respectively.

2) TDS (Total Dissolved Solids)

TDS of the sample was calculated by two different methods:

a) By using a TDS meter (in ppm)

b) Through gravimetric method (in mgL^{-1})

A clean Petri dish was subjected to a temperature of 100 ˚C in an oven, cooled in a desiccator and then weighed to constant weight. The collected wastewater sample was filtered into a clean conical flask using a pre-weighed filter paper. A known volume of the filtrate was poured into the petri-dish and heated in an oven at temperature 180 ˚C. The obtained residue was then cooled in the desiccator and weighed to a constant weight. The TDS (in mgL^{-1}) was calculated with the formula below:

$$TDS = \frac{(A - B) \times 1000}{Volume\ of\ sample(ml)}$$

Where, A = weight of dried residue + evaporating dish (mg); B = weight of the evaporating dish (mg).(Aniyikaiye.T *et al*, 2019), (Bhat *et al*, 2018)

3) DO (Dissolved Oxygen)

The estimation of DO is done by titrimetric method. The oxygen of the water combines with manganous hydroxide, which on acidification liberate iodine equivalent to that of oxygen fixed. This iodine is titrated by standard sodium thiosulfate solution using starch as an indicator. The dissolved oxygen content of sample was calculated (in mgL^{-1}) by applying following equation:

$$DO = \left(\frac{8 * \times 1000 \times N}{V}\right) \times v$$

Where, V= volume of sample taken (ml); v= volume of titrant used; N= normality of the titrant; *= 8 is the constant since 1ml of 0.025 sodium thiosulfate solution is equivalent to 0.2mg oxygen.

4) BOD (Biochemical Oxygen Demand)

The Biochemical Oxygen Demand (BOD) is a way of expressing the amount of organic compound in sewage as measured by the volume of oxygen required by bacteria to metabolize it under aerobic conditions.pH of the sample was adjusted to neutrality by using 1N acid or 1N alkali solution. Water sample was filled in 6 BOD bottles carefully without bubbling followed by the addition of 1ml of allylthiourea to each bottle. Dissolved oxygen content was determined in 3 out of 6 BOD bottles by the titration method and mean readings were calculated (D_1). Rest of the 3 BOD bottles were incubated at 27 ˚C in a BOD incubator for 3 days. The oxygen concentration were determined in all the 3 incubated samples and mean readings were recorded (D_2). BOD of the sample was calculated by using formula below:

$$BOD\ (mgL^{-1}) = D_1 - D_2$$

Where, D_1= initial DO in sample (mgL^{-1}); D_2= DO after 3 days of incubation (mgL^{-1})

5) COD (Chemical Oxygen Demand)

Determination of COD in water mainly involves the reaction of the water sample with strong oxidizing agent which oxidizes the organic matter in it. COD of the wastewater sample was obtainedthrough open reflux method. This was carried out by the addition of mercuric sulphate and sulphuricacid into an aliquot of wastewater sample in a reflux flask. On cooling, the obtained solution wasreacted with known concentration of potassium dichromate and known volume of sulphuric acid.The solution was refluxed for 2 h and cooled. The obtained solution was diluted to twice its volume,cooled to room temperature and excess K2Cr2O7 in it determined by titrating with ferrous ammonium sulphate using ferroin indicator. Similarly, a blank with all reagents added to 25 mL of distilledwater was titrated:

$$(mgL^{-1})COD = \frac{(A-B) \times C \times 8000}{Volume\ of\ the\ sample(ml)}$$

Where, A = volume of titrant used for the sample (mL); B = volume of titrant used for the blank sample (mL); C = the normality of the ferrous ammonium sulphate.(Aniyikaiye.T *et al*, 2019), (Usharani K *et al*, 2010)

6) Chloride Test

Concentration of chloride ions sometimes increases in water, for safety reasons chloride in wastewater should not exceed 350 mg/L as directed by FME and WHO Standard. For chloride test 50ml of sample was taken in a conical flask followed by the addition of 2ml of $K_2Cr_2O_7$. 0.025N $AgNO_3$ solution was poured into burette set with titration assembly. The sample was then titrated with $AgNO_3$solution until reddish tinge appears. Amount of chloride ions (in mgL^{-1}) in the water sample was calculated by using formula below:

$$Chloride = \frac{Volume\ of\ AgNO3 \times 1000 \times 35.5}{\text{Volume}\ of\ water\ sample\ used}$$

7) Nitrate Test

Nitrate in effluent was determined by using UV Spectrophotometric method.

8) Electrical Conductivity (EC) Test

The EC of the effluent sample were carried out with an aid of a salinometer (Aniyikaiye.T *et al*, 2019).

C. Microbial Analysis

Media Preparation

1) *For Bacteria:* Nutrient Agar Media (NAM) was prepared by mixing peptone (5.0g), beef extract (3.0g), NaCl (5.0g) and agar (15.0g) in distill water (1L) and sterilized in autoclave at 15lb pressure for 15min.
2) *For Fungus:* For Czapek-Dox agar sodium nitrate (2.0g), magnesium sulphate (0.5g), potassium chloride (0.5g), ferrous sulphate (0.01g) was dissolved in half of the water followed by the addition of sucrose (30.0g) and agar (15.0g). Dipotassium hydrogen phosphate (1.0g) was dissolved separately and added to the rest making volume to 1L. Media was then sterilized by autoclaving at 121°C for 15minutes.
3) *Isolation and Identification:* Sample effluent was taken into 6 test tubes and were serially diluted from 10^{-1} to 10^{-5} whereas one was left blank as control. The sample from each test tube was then streaked on NAM and Czapek-Dox petri plates with the help of inoculation needle under aseptic conditions followed by incubation at 37°C for 24 hours in case of bacteria and for fungal growth. Bacterial identification was done on the basic of Gram staining and Acid fast staining whereas for fungus Lactophenol cotton blue mounting procedure was performed.

III. RESULT AND DISCUSSION

Parameters	Unit	Obtained Value
Color	-	slightly brownish and turbid
Temperature	°C	36.6
pH	-	7.9
TDS(by TDS metre)	ppm	486
TDS(Gravimetric method)	mgL^{-1}	710
DO	mgL^{-1}	8.0
BOD	mgL^{-1}	188.72
COD	mgL^{-1}	326.16
Chloride	mgL^{-1}	284
Nitrate	mgL^{-1}	41.26
Conductivity	mSm^{-1}	138.16

Table:1- Physiochemical analysis of waste water of food industry effluent of kuthrel area, Durg district.

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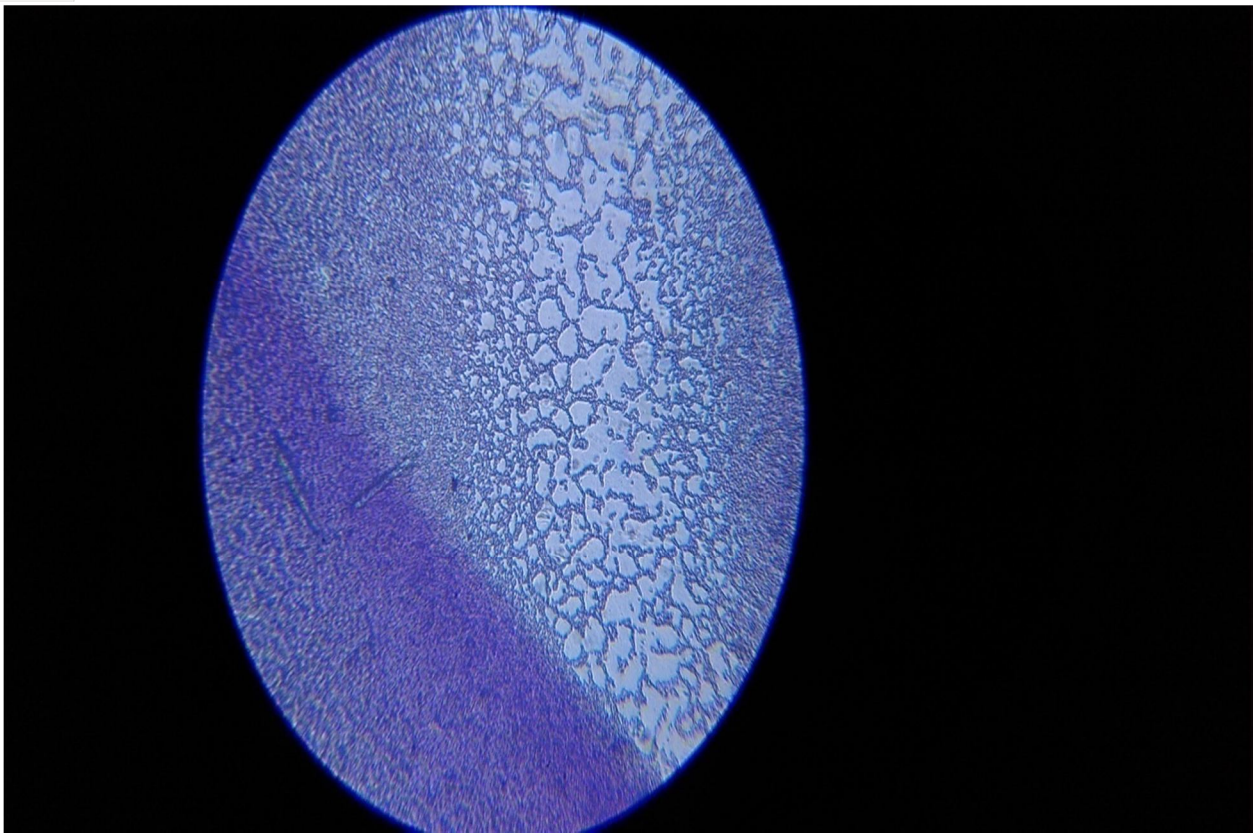


Fig:1-Slides were observed under 100x magnification after gram staining

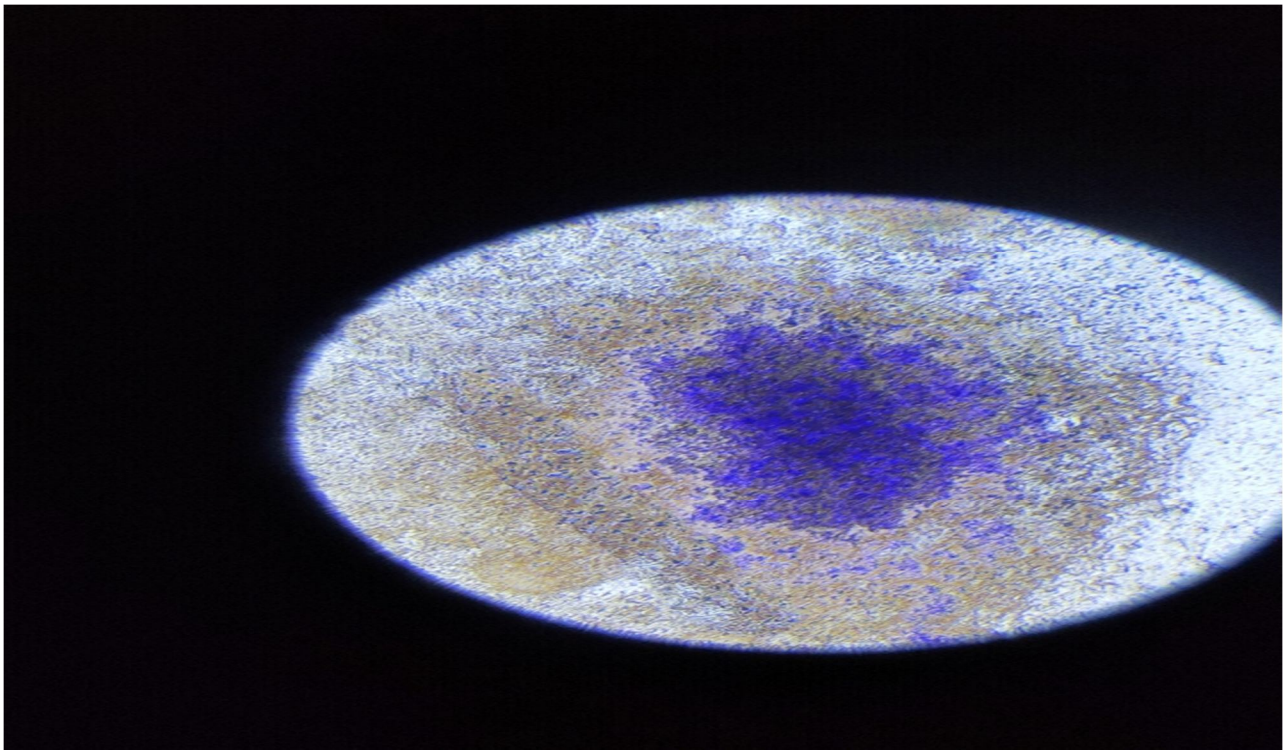


Fig:2- Slides were observed under 100x magnification after acid fast staining

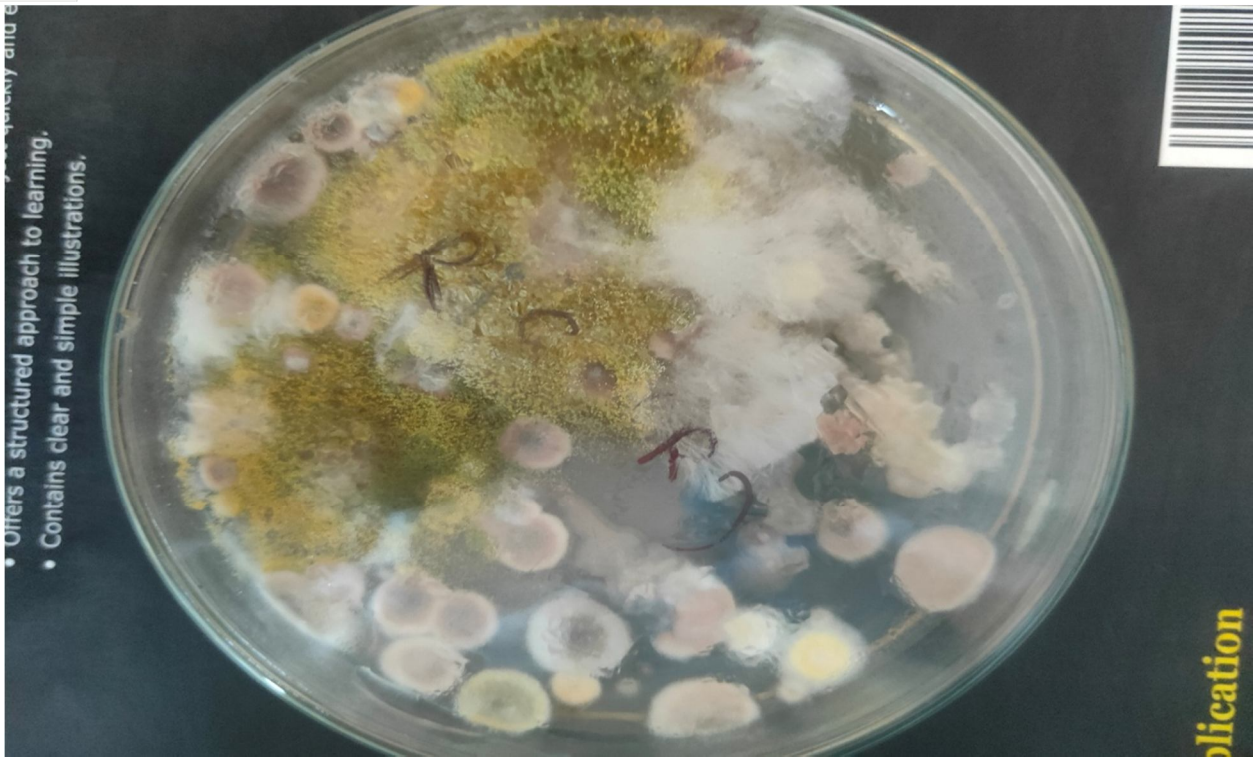
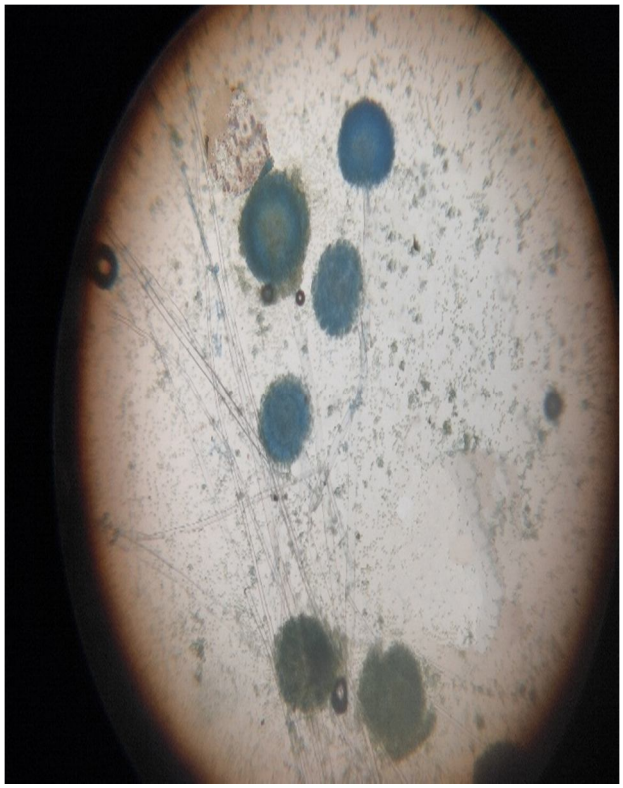
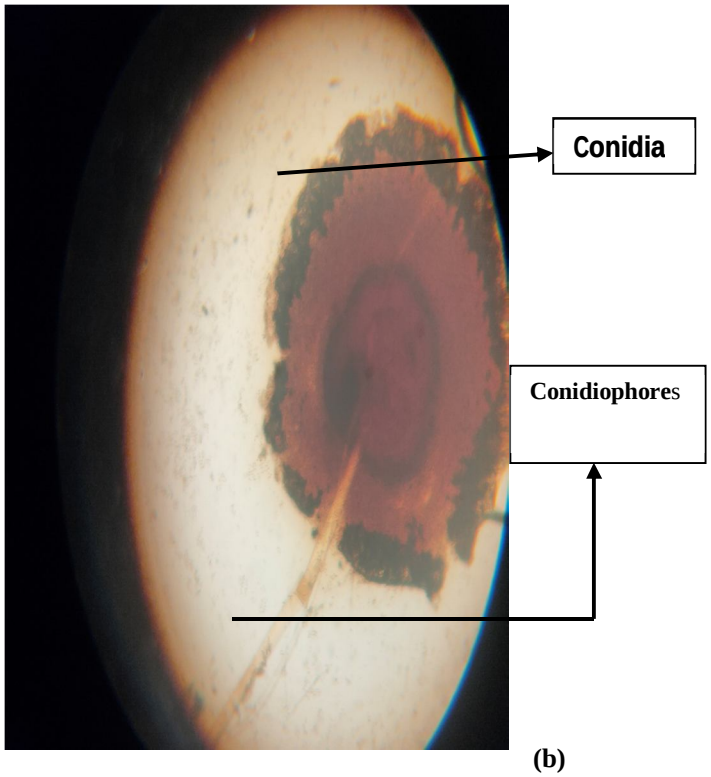


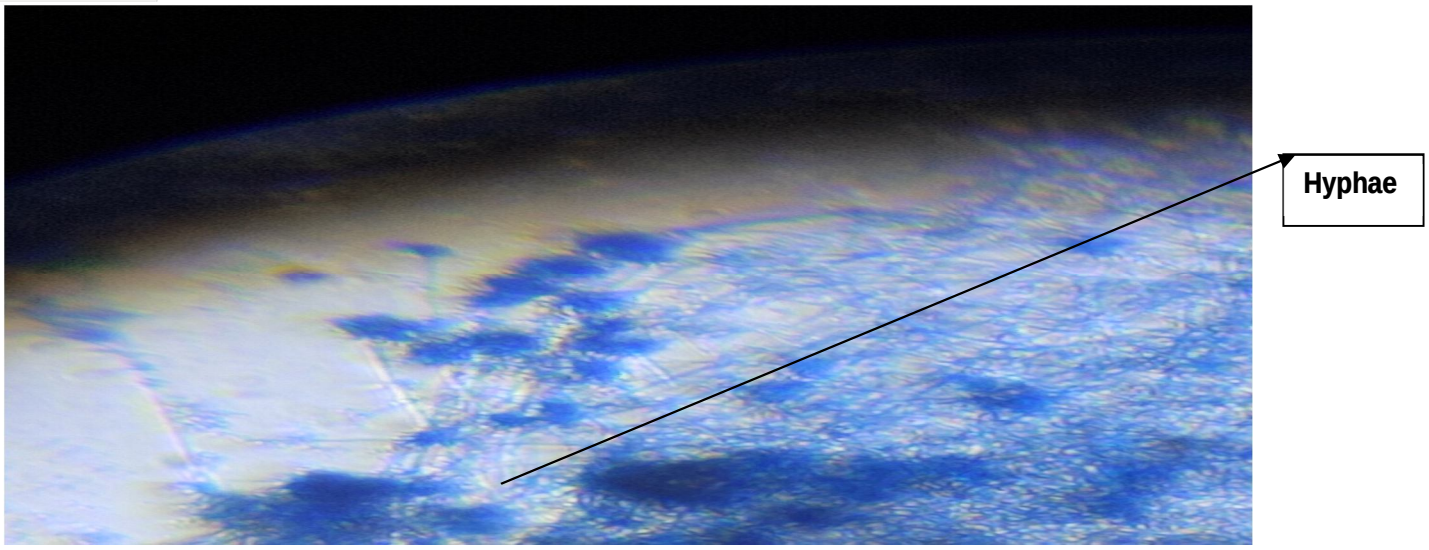
Fig:3- shows results of fungal growth on Czapek Dox Agar plates



(a)



(b)



(c)

Fig:4-Lactophenol cotton blue mounting of fungi

A. Physiochemical Analysis

In present study the physiochemical parameters and microbial analysis of waste water of food industry effluent was carried out to study the nature and pollution level of the industrial effluent.The effluent color was observed as slightly brownish and turbid. Temperature was recorded as 36.6 $^{\circ}$C during the time of sample collection. The pH of the sample was 7.9 suggesting slightly basic nature of the effluent. Value of Total Dissolved Solids (TDS) was measured by two different methods: first by using TDS meter which showed the value of 486ppm and second, through Gravimetric method which gave the TDS value of 710mgL^{-1}. However the readings of Dissolved Oxygen (DO) obtained was a bit low i.e. 8.0 mgL^{-1}. Low DO concentration in effluent is an indication of high microbial activities in the water due to presence of biodegradable organic compounds in the effluent (Aniyikaiye.T *et al*, 2019). The value of Biochemical Oxygen Demand (BOD) was calculated as 188.72 mgL^{-1} which was under the standard range for discharge according to Central Pollution Control Board (CPCB) i.e. 30-200 mgL^{-1}. High BOD concentration reduces oxygen level in water thereby depleting its availability for aquatic life forms resulting in their death. COD concentration was determined as 326.16 mgL^{-1}, which suggested the presence of high amount of organic matter in the effluent. Whereas the values of chloride and nitrate in the sample were calculated as 284 mgL^{-1} and 41.26 mgL^{-1} respectively. Readings of Electrical Conductivity obtained was 138.16 mSm^{-1} suggesting the presence of inorganic ions in the water. Table:1 shows the value of physiochemical parameters recorded.

B. Microbial Analysis

The results in fig:1 shows most of the bacterial cells were pink indicating that the effluent had high concentration of gram negative bacteria than gram positive. Moreover, acid fast staining results suggested higher levels of non-acidic bacteria fig:2. Some colonies on Czapek Dox agar plates were white (*Aspergillus versicolor*) while some were white initially and became yellow later on *(Aspergillus flavipes),* fig:3. Conidiophores septate developed as stalk and head form footcells producing conidiophores at long axis fig:4(b).Vegetative mycelium septate was branched and hyphae was colorless fig:4(c). Microbial analysis suggested high concentration of microbial population in the food industry effluent which clarifies low DO and high BOD levels. Increased microbial population will affect aquatic life forms present in the water bodies into which the effluent might get discharged furthermore, it might also cause illness among stray or domestic animals who might drink it. Complete test for coliform bacteria can also be performed so as to identify the type of bacteria present in the effluent.

IV. CONCLUSION

Industrialization in India has been leading the country towards the development. However its waste water analysis has been an intriguing subject in recent years because of issues related to water pollution. Present study intends to determine the physiochemical and microbial analysis of waste water of food industry effluent. The experimental data suggested that the effluent is slightly basic in nature with pH 7.9 whereas, low value of DO and high value of BOD and COD were observed indicating high microbial population

and presence of organic matter. Although the value of BOD obtained was within the standard range of discharge according to CPCB but the microbial load present in the effluent might lead to the contamination of water bodies into which it might get discharged. Adequate and proper treatment of the effluent is necessary before its discharge so as to reduce the microbial load thereby reducing water pollution.

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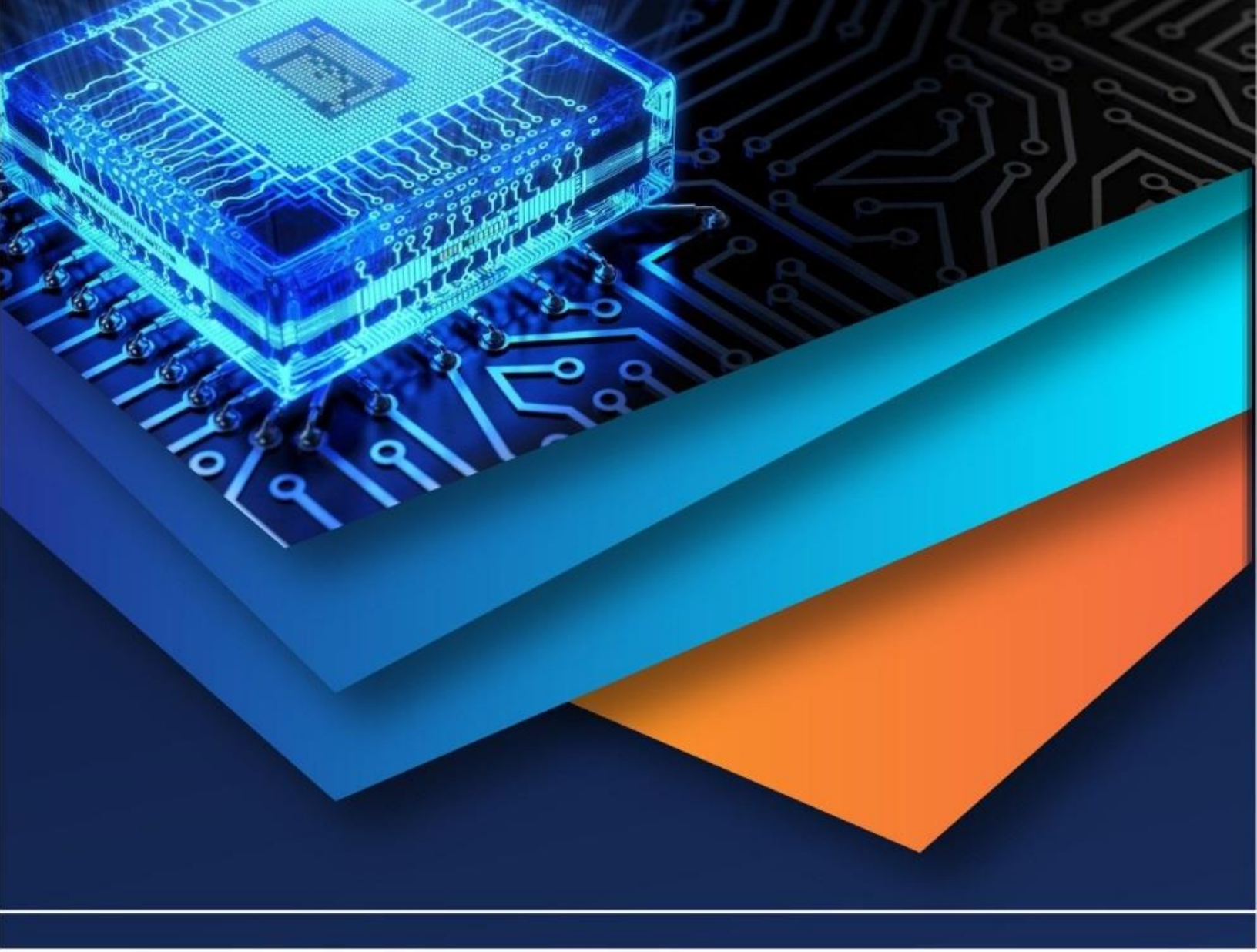

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