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Performance of Individual Isolates in the Reduction of Sulphide Ions, BOD and COD Parameters in the Treatment of Unhairing/Liming Tannery Wastewater

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ABSTRACT

Five anaerobic microorganisms were isolated from aged sulphide-lime unhairing tannery wastewater following conventional methods. They were characterized and identified as gram positive bacilli. The same isolates were used for the treatment of similar tannery wastewater after pretreatment (filtration, heating/sterilization, covered, allowed to cool overnight). The procedure was repeated three consecutive days to minimized trace of microorganisms and adjusted to pH 7.0 using diluted HCl. To each 150 ml plastic container, 100ml of the pre-treated wastewater was added and each isolate was inoculated into the sample for treatment within the periods of 5, 7 and 9 days, at different temperatures of 22 ^{o}C, 27 ^{o}C and 37^{o}C with 0.5 and 1.0 McF standards accordingly. The treated wastewater was analyzed for reductive performance of individual Isolates on pollutants which was determined by sulphide ions, BOD and COD parameters. It was generally observed that increasing period of treatment, McF standard of the microorganism's and temperature indicated general reduction of the measured parameters. Reduction of sulphide ions became increasingly less for virtually all the isolates contrary to the higher percentage reduction from the beginning. This suggests increase in depleting of the organic matter. However, T_{I}, T_{II} and T_{III} appear to be more effective in the reduction of sulphide ions. Isolates T_{III}, T_{IV} and T_{V} consistently reduced COD in the wastewater more than other isolates in this order: 68.33%, 69.6 % and 71.4% respectively. T_{III}, T_{II} and T_{IV}, gave the highest percentage reduction of BOD at 67.94%; 65.0% and 69.84% respectively, from the treated sulphide-lime unhairing tannery wastewater.

INTRODUCTION

There has been a growing interest regarding anaerobic treatment of tannery wastewater due to the several advantages over other methods. The method is gaining popularity over the aerobic as it is more effective, requires less energy and less maintenance cost [1-3]. The method requires microorganisms that do not require oxygen for their respiration; rather they use other sources of oxidation to abstract energy for their existence. In anaerobic treatment electrons donating substances like sulphate and carbon are often used as energy sources. Tannery wastewater which consists of many organic carbons is capable of being used as a source of carbon donating electrons to sulphur which is reduced [2, 4a, 4b]. By this oxidation-reduction, the microorganisms' abstracts energy, consequently the organic materials are depleted and the pollutants in the wastewater gradually get reduced by degradation [5].

This method of tannery wastewater treatment is however associated with some drawbacks which include: i) The continuous production of hydrogen sulphide gas during anaerobic treatment discourages the implementation of the method, resulting from the reduction of sulphate which occurs in the absence of alternative electron acceptors like (oxygen and nitrate). ii) High protein component in the wastewater affects selection of biomass, slow kinetics of hydrolysis, inhibits granular sludge formation [6, 7].

Different methods of wastewater treatment have been conducted; each is associated with its challenges and prospects [8, 9]. Oxidation-coagulation-filtration processes for the reduction of sulphide from the hair burning liming wastewater in tannery requires a lot of energy, but can remove sulphide by means of oxidation up to 95% before coagulation [10, 11]. It has also been reported that excellent reduction of pollution load in a highly concentrated tannery wastewater by electrocoagulation using impact quality aluminium as electrode [12-14].

Some methodologies may not be economically feasible because of high cost or expertise required for implementing and sustaining the operation of such processes [15, 16]. The potential environmental impact of chemicals used in the treatment of this complex tannery effluent has been widely acknowledged and some reagents used for the methods of treatment are more toxic than threats posed by these effluents. Hence, anaerobic treatment of tannery wastewater is observed as the ultimate in term of cost effectiveness.

The aim of this study therefore is to compare the performance of each isolate on the reduction of pollutants determined by COD, BOD and sulphide ions which poses serious challenge in tannery wastewater management.

METHODOLOGY

Anaerobic Treatment of the Tannery Wastewaters with Different McFarland Standard

The acidity of filtered tannery sulphide-limed wastewater was adjusted to pH 7.0 using diluted HCl (1:1). The wastewater was heated to 70 °C and the temperature maintained for fifteen minutes, covered and left overnight. This was repeated for three consecutive days. Then 500 cm^3 was reserved as CTL sample and the remaining distributed into 120 cm^3 plastic bottles each containing 100 cm^3 of filtered sulphide-lime unhairing wastewater. Then 0.5 cm^3 of sodium alginate was added and shaken to prevent the sampled wastewater from settling. Triplicate sample wastewater were inoculated with each isolate, according to standard procedures as reported by Pepper and Gerba [17] and incubated anaerobically at 22 °C, 27 °C and 37 °C. Then COD, BOD_5 and S^{2-} ions were determined at Day 5, 7 and 9 days, to estimate the reduction of each pollutant determined by the parameters.

Analysis of the Tannery Wastewaters

Analyses of COD, BOD and sulphide ions concentration of both control and samples treated wastewater were conducted according to standard procedures as reported by USEPA **[18]** for wastewater assessment and monitoring.

Determination of Biochemical Oxygen Demand (BOD)

The azide modification of the Winkler method was used as reported by USEPA [18]. Sample waste water (0.5 cm^3) was measured into a 300 cm^3 BOD bottle. Then 2.0 cm^3 of manganese sulphate solution and 2.0 cm^3 of alkaline iodide-azide reagent were added to the sample and carefully filled with distilled water. The BOD bottle was stoppered to exclude air bubbles and mixed by inverting the bottle several times. The BOD bottle with the content was kept on the table, until the precipitates were settled, leaving a clear supernatant. The bottles were shaken again and allowed to stand for a minute. Then 2.0 cm^3 of $H_2SO_4$with specific gravity of 1.86 g/$cm^3$was added into the mixture and inverted 3 times and allow standing for the precipitate to settle. Then, 200 cm^3 of supernatant was measured into 500 cm^3 conical flask and 1.0 cm^3 of starch indicator was added and titrated with 0.025M of $Na_2S_2O_3$ solution to a pale straw colour. The dissolved oxygen (DO) in each wastewater sample was calculated on the basis that 1.0 cm^3 0.025 M sodium thiosulphate titrant is equivalent to 0.2 mg DO. Thus each centilitre of sodium thiosulphate titrant used was equivalent to 1mg/dm^3 DO. The same procedure adopted for DO was used to determine (BOD_5) except for the five days' incubation period. The same 0.5 cm^3 volume of sample was placed in a 300 cm^3 BOD bottle and gently filled with water. The bottle was stoppered with no air bubbles and incubated for a period of 5 days in the dark at 20±2°C. The DO was measured before and after. Then BOD_5 calculated from the difference between initial and final DO using the expression below:

$$\text{BOD as} \left(\frac{mgO_2}{L}\right) = \frac{D_O - D_5}{p}$$

Where, Do = the DO of the diluted sample solution (mg/dm^3); P = the decimal dilution factor

D_5 = the DO of diluted sample after 5-day incubation (mg/dm^3)

Determination of Chemical Oxygen Demand (COD) - Open Reflux Method

For COD determination, 20 cm^3 of diluted sample wastewater (0.5:100) was measured into a 500 cm^3 conical flask. Then 0.4 g of $HgSO_4$ mixed with 10 cm^3 standard $K_2Cr_2O_7$ solution and a few pieces of anti-bump granules were added into the flask and placed on a hot plate. The flask was then attached to the reflux condenser and 30cm^3 of H_2SO_4 with specific gravity of 1.86 g/cm^3containing $AgSO_4$ was slowly added into the flask and mixed thoroughly. The mixture was then refluxed for one hour, cooled and the condenser rinsed with 25 cm^3 water. Three drops of ferroin indicator was added and the mixture titrated with standard ferrous ammonium sulphate ($Fe(NH_4)_2SO_4$) until a sharp colour change from blue-green to reddish brown was observed. A blank was prepared and process repeated with all the reagents except the sample. The COD in each sample was calculated using the expression:

$$\text{COD as} \left(\frac{mgO_2}{L}\right) = \frac{(A - B)M \times 8000}{\text{ml of sample taken}}$$

Where, A = Volume of $Fe(NH_4)_2SO_4$ used for blank

B = Volume of $Fe(NH_4)_2SO_4$ used for sample

M = Molality of $Fe(NH_4)_2SO_4$ used in titration.

Determination of Sulphide (S^{2-}) – Iodometric Method

The sample wastewater was diluted with distilled water in the ratio of 1:20 and thoroughly mixed in a 500 cm^3 beaker and 200 cm^3 of the mixture was withdrawn into a 500 cm^3 conical flask. Then 2.0 cm^3 of standard iodine solution together with 2.0 cm^3 6.0 M HCl was gently added after shaken. 1.0 cm^3 Starch indicator was added and thoroughly mixed by shaking and titrated with sodium thiosulphate (0.025 M) until the solution turned light brown. Then S^{2-} concentration in each sample was calculated using the expression:

$$Sulphide\left(\frac{mg}{L}\right) = \frac{(A \times B) - (C \times D) \times 16000}{volume\ of\ sample}$$

Where:

A= Volume of standard iodine solution

B = Molarity of standard iodine solution

C = Volume standard $Na_2S_2\,O_7$ titrant

D = Molarity of standard $Na_2S_2O_7$ titrant

RESULTSAND DISCUSSION

Table 1.0: Mean Reduction of the Parameters by Different Isolates After treatment

TREATMENT	N	COD	BOD	Sulphide
		Mean ± SD	Mean±SD	Mean±SD
CONTROL	18	28833.3±923.55[a]	1280.0±24.49[a]	580.7±11.94[b]
TI	36	14526.9±3452.10[bc]	836.9±292.31[b]	626.7±63.98[a]
TII	36	14283.1±3731.01[c]	803.1±254.26[b]	615.2±62.62[a]
TIII	36	13761.1±2970.51[cd]	837.8±256.74[b]	625.3±57.42[a]
TIV	36	13072.8±2948.92[d]	812.4±259.67[b]	618.1±46.52[a]
TV	36	15363.9±3279.52[b]	1244.4±1764.83[a]	617.6±47.48[a]
Total	198	15531.7±5292.11	940.8±800.95	617.0±54.39

Notes: Means with the same letter are not significantly different

Table 2: Two Sample t-test on Effects of Different McFarland Standard on Pollutants

Parameter	McF	N	Mean	Std. Error	t-value	DF	P-value
COD (mg/L)	0.5	99	16142.8	476.773	1.632	196	0.104
	1	99	14920.6	577.701			
BOD (mg/L)	0.5	99	963.24	92.063	0.393	196	0.695
	1	99	918.4	67.382			
SULPHIDE (mg/L)	0.5	99	624.56	4.471	1.981	196	0.049
	1	99	609.35	6.237			
TDS (mg/L)	0.5	99	26130.9	339.45	1.604	196	0.11
	1	99	27251.4	610.387			
TSS (mg/L)	0.5	99	10473.1	293.081	0.231	196	0.818
	1	99	10590.5	415.087			
E. COND (µS/cm^3)	0.5	99	20257.4	927.791	0.901	196	0.369
	1	99	18911.1	1170.79			

ANOVA for Anaerobic Treatment of the Sulphide-lime Unhairing Wastewater

Three parameters, BOD, COD and S^{2-}concentrations, were considered. The microorganisms coded as T_I, T_{II}, T_{III}, T_{IV} and T_V were used for the treatment of unhairing/liming wastewater for its degradation. The parameters were also used to measure the individual performance of the isolates. The data collected were analyzed with the General linear model (GLM) to assess the level of reduction of pollution by the isolates at different McFarland standards, temperatures and periods of treatment. Means obtained from analysis of variance (ANOVA) along the post hoc test on the means using Duncan Multiple Range Test (DMRT), of effectiveness in pollution reduction are indicated in alphabetic letters in descending order Table I. The computation takes into considerations all temperatures, different McFarland standard and period of treatment.

Performance of Individual Isolates on BOD Reduction

For BOD, CTL sample (1280.0±24.49 mg/L) was significantly different from samples treated with other isolates except for T_V (1244.4±1764.83 mg/L) $p<0.05$. However, between the individual isolates, no significant difference was observed in their reducing effect ($p> 0.05$). Treatment of the wastewater with 0.5 McF at 22 °C for five days achieved a reduction of 23.28% of BOD with T_I. On Day 7 further reduction of BOD by isolate T_I was only 38.09% at 37ºC. which is in line with previous findings [19]. At the end of Day 9, maximum reduction in BOD of 71.43% was noted by isolate T_V at 37°C with 0.5McF (Figure III). The results of Day 9 treatment period further support the influence of temperature and time on BOD.

The increase of McFarland standard from 0.5 – 1.0 did not have much influence on percentage reduction of BOD, as shown in Figure IV. BOD increased only slightly by 34.93% with isolates T_V at 37°C with 1.0 McF treatment. However, reduction in BOD increased generally when treatment period was extended (Fig.I - III and Fig. IV - VI). On Day 9 the percentage reduction of BOD was observed to increase particularly for $T_{III,}$ T_{II} and T_{IV} (Fig. IV - VI), giving the highest percentage reduction of BOD at 67.94%; 65.0% and 69.84% observedat temperatures of 22 ºC, 27 ºC and 37 ºC respectively with 1.0 McF (Fig.VI). This implies that Isolates $T_{III,}$ T_{II} and T_{IV} indicate the potentials of being able to degrade organic matter in sulphide/lime unhairing wastewater more effectively along with others with similar tendencies.

Individual Isolates on COD Pollutants Reduction

The ANOVA indicates mean levels for COD and CTL (28833.3±923.55mg/L) samples, had the highest concentration and were significantly different from samples treated with the isolates, T_I - T_V (P< 0.05). Samples treated with T_{IV} (13072.8±2948.92 mg/L had the least level of COD and significantly different from the level of samples treated with T_{III}. Samples treated with T_V and T_I, were not significantly different (P>0.05) and had the least reducing effect of the pollutant. In the overall assessment, T_{IV} (13072.8±2984.92 mg/L) had the best reducing effect of the pollutants determined by COD parameter followed by T_{III} (13761.1±2970.51 mg/L).

Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Concentration at Different Conditions of Treatment.

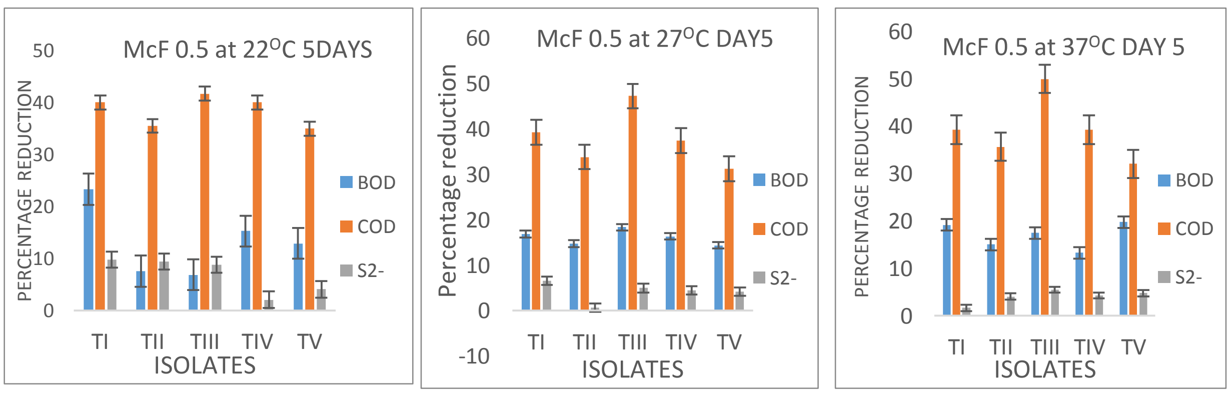


Figure I: Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Conc. With McF 0.5 for Day 5

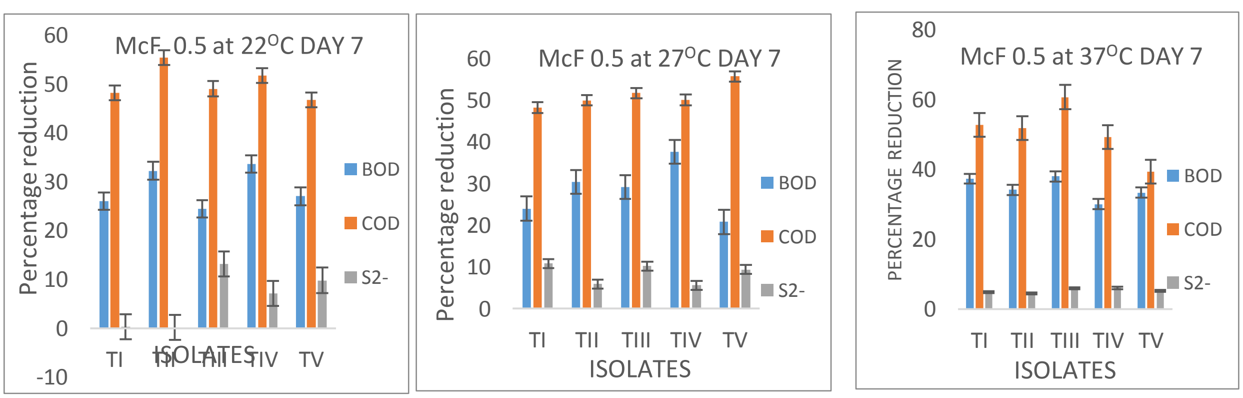


Figure II: Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Conc. With McF 0.5 for Day 7

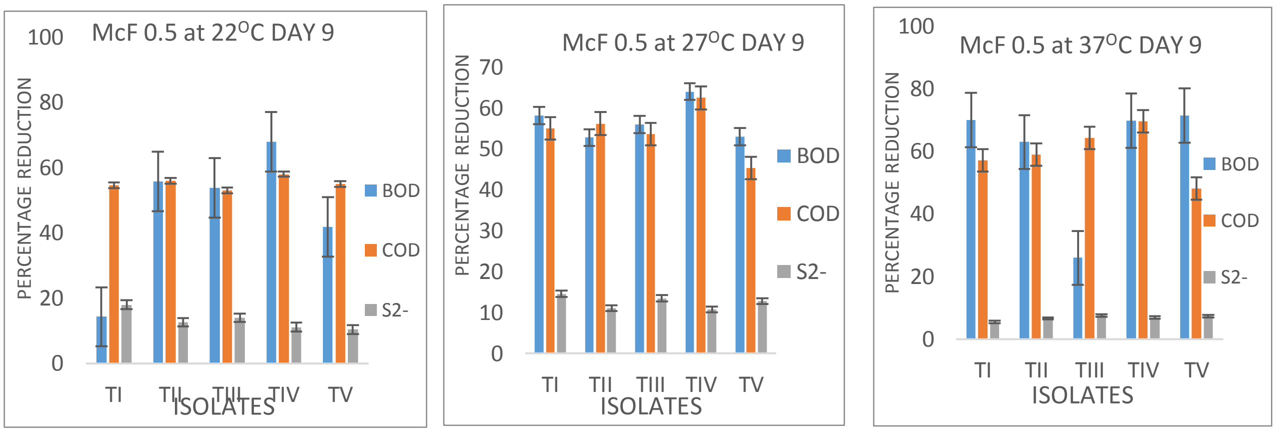


Figure III: Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Conc. With McF 0.5 for Day 9

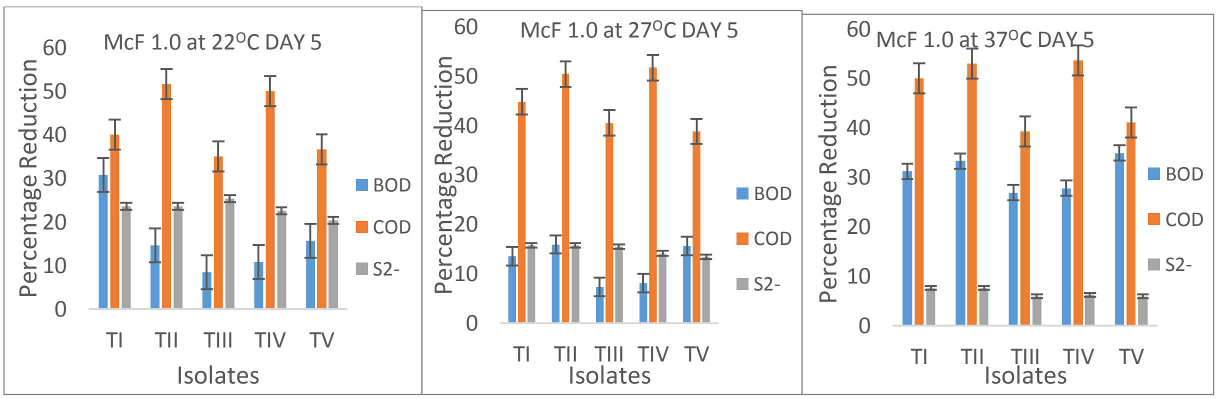


Figure IV: Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Conc. With McF 1.0 for 5 Days

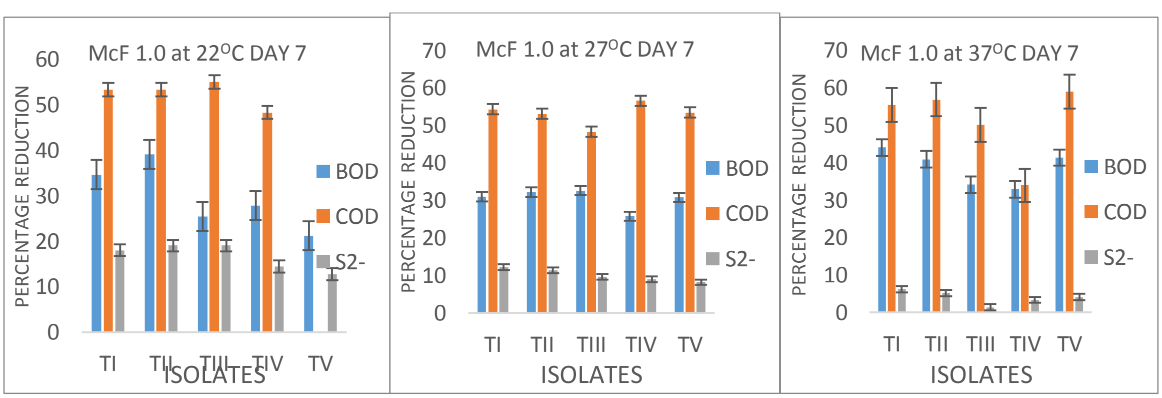
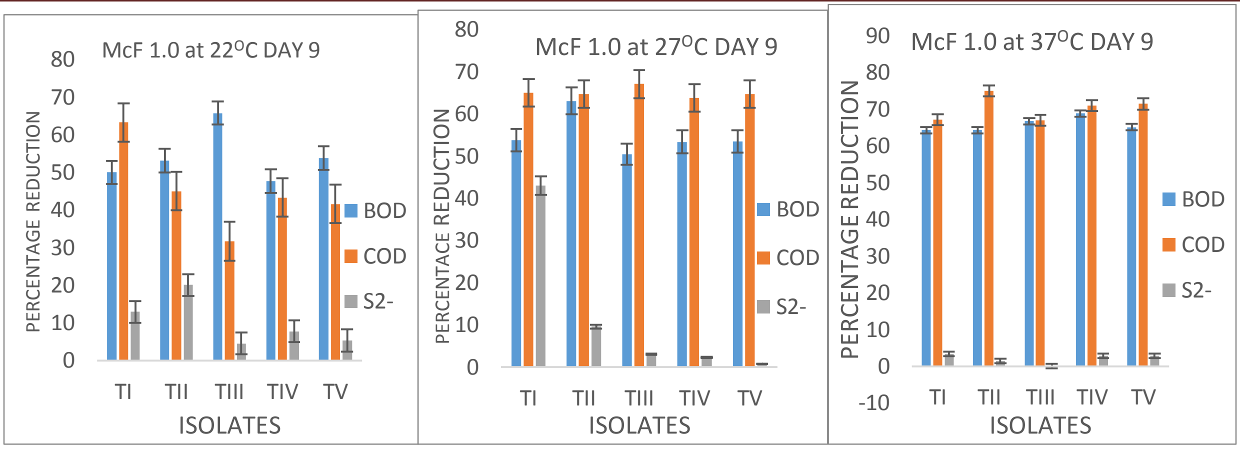


Figure V: Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Conc. With McF 1.0 for 7 Days



` Figure VI: Reductive Performance of Individual Isolates on BOD, COD and Sulphide Ions Conc. With McF 1.0 for 9 Days

The anaerobic isolates were used for the degradation of sulphide /lime unhairing wastewater at varied temperatures, time and McFarland standards. The level of degradation of pollutants on the wastewater was analyzed via the following parameters: BOD, COD and S^{2-} Ions concentration. Statistical analysis of the two sample t-test of anaerobic treatment results shows various P-values of the pollution parameters but, only sulphide ions concentration was significantly different at ($P < 0.05$),

Figure I.0 reflects the effects of isolates on unhairing-lime wastewater pollutants in the reduction of COD. An increase in percentage reduction of COD was observed for T_{III} by 51.76 % and 60.71% reduction at, 27 °C and 37 °C respectively after day 7 treatment periods (Figures.1.0 - III). On Day 9 treatment period isolate T_{IV} considerably reduced COD to 69.6 % with 0.5McF at 37 °C. Increasing McFarland standard from 0.5 – 1.0 McF, further percentage reduction in COD was observed. On Day 5 treatment period, isolate T_{II} had 51.7% reduction at 22°C followed by isolate T_{IV} having 51.72% and 53.6% at 27°C and 37°C temperatures respectively. Similarly, after day 7 treatment period further reduction in COD was observed by most isolates particularly isolates T_{IV} and T_V which showed reduction by 56.6 % at 27° C and 56.8 % at 37 °C (Fig. V).

For Day 9 treatment period, isolate T_{III} caused COD reduction by 68.33% at 22°C with 1.0 McF standard. Isolates T_{IV} also reduced COD in the wastewater by 63.8% at 27°C while T_V by 71.4% at 37 °C. The trend suggests that temperature and treatment period influence COD reduction in the wastewater.

Comparing the performance of individual isolates on COD response, it was observed that after Day 9 treatment periods, isolate T_{IV} indicated a reduction of COD by 69.6%, the highest percentage reduction observed with 0.5 McF standard at 37 °C. While, for the same period at 1.0 McF, 68.33 % reduction was observed at 22 °C, followed by 64.7% triggered by isolate T_{II} and T_V reduction was recorded for isolate T_{III} at, 27° C. The steady reduction of COD observed could be attributed to the oxidation of carbon and other organic matter from the wastewater induced by the anaerobes. The process also led to the generation of hydrogen sulphide that was observed given off. This might have contributed to further reduction in COD from the wastewater which is in line with reports by Tilley et al [20]. The evolution of hydrogen sulphide gas observed, suggests that the microbes isolated were sulphur reducing anaerobic bacteria [21]. This may account for the general increase observed in percentage reduction of COD from the wastewater, as sulphide was observed evolving in form of H_2S. From the overall results it can be stated that isolates T_{III} and T_{IV} consistently reduced COD parameter in the wastewater more than other isolates.

Hydrogen sulphide

The evolution of sulphide (S^{2-}) observed from the anaerobic treatment of sulphide lime unhairing wastewater may likely pose negative effects, similar to reports by Khanna and Lofrano [22]. This will give bad odour and poses serious health threats to tannery and wastewater treatment plant workers. It was observed that the effects of anaerobic isolates caused reduction of sulphide in the wastewater, though the results were observed to fluctuate with different isolates at different conditions of treatments. After day 5 treatment periods, T_I had maximum percentage reduction of 9.75% at 25 °C which declines to 6.58% at 27 °C and 1.72% at 37 °C with 0.5 McF.

Similar observations were made for other isolates like T_{III} reduced sulphide by 13.05%, at 25 °C. Isolate T_{IV} had maximum percentage reduction of sulphide concentration by 6.03 % at 37 °C after 7days of treatment. On increase of McFarland standard from 0.5 – 1.0 McF, consistent reduction in sulphide (S^{2-}) was observed through all the temperature regimes and period of treatment (Figures IV - VI). The maximum percentage reduction of sulphide pollutants was 25.35% for T_{III} and 23.6% for both T_I and T_{II}. This was observed after 5 days of treatment with 1.0 McF at 22 °C. But for the same isolates, reduction of sulphide was much less at 37 ºC for the same 5days as shown in Figure IV. This is most probable because the carbon content of the wastewater that induced the reduction of sulphur may be depleting. This observation agrees with the literature reported by Lofrano *et al* [23].

CONCLUSION

It was generally observed that increasing period of treatment, temperature and the McF standard of the microorganisms favored reduction of the measured parameters, especially as the isolates seemed to be mesophilic in nature. Reduction of sulphide ions was observed becoming less for virtually all the isolates however, T_{I}, T_{II} and T_{III} appears to be more effective in its reduction. The reduction of sulphide also positively affected the reduction of COD as sulphide concentration could cause high COD. From the overall results it can be stated that isolates T_{III}, T_{IV} and T_{V} consistently reduced COD in the wastewater more than other isolates in this order, 68.33%. 69.6 % and 71.4% accordingly, suggesting these isolates as most effective compared to others. While, for T_{III}, T_{II} and T_{IV}, they gave the highest percentage reduction of BOD at 67.94%; 65.0% and 69.84% in that order.

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