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ANAEROBIC TREATMENT OF SULPHIDE-LIME UNHAIRING AND LIMING TANNERY WASTEWATER AND ITS EFFECTS ON COD AND BOD PARAMETERS

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ABSTRACT
Tannery sulphide-lime unhairing and liming wastewater is known to be highly polluted with very high values of COD and BOD and many other pollutants that poses serious threat to land and aquatic systems when discharge without treatment. Five anaerobes were isolated and identify as gram positive, they were inoculated into the sulphide–lime unhairing wastewater for treatment, at room temperature, 27°C and 37°C for 5,7 and 9 days with different McFarland standards of 0.5 and 1.0 McF. The results obtained shows various percentage reduction of COD and BOD. Considering the effectiveness of individual isolates, it was observed that at day 9, the highest percentage reduction of BOD_5 was recorded by isolate T_{IV} at 67.94 %; 65.0 % and 69.84 % at room temperatures, 27°C and 37°C respectively with 0.5 McF, implying that the isolates are likely to be mesophilic (35°C – 37°C). Further reduction of COD was recorded when treatment temperature and time was increase to 37°C such that after day 9, isolate T_{IV} indicated a reduction of 62.5 % and 79.29 %, at 27°C and 37°C respectively. The maximum reduction of COD with 1.0 McF at 37°C for day 9 treatment period was 69.91 % by isolate T_V. The mechanism is based on the microorganism which induced oxidation of carbon content of the wastewater abstracts energy through the process. The process also caused the reduction of Sulphur present in the wastewater leading to the generation of hydrogen sulphide gas which suggest the source of smell of the gas in any tannery lime yard.
Key words: ***Sulphide–lime, Wastewater, Microorganisms, Treatment***

INTRODUCTION

Large volumes of effluent and pollutants from the leather manufacturing industry contaminate surface and underground water if discharged without treatment. The high concentration of pollutants with low biodegradability in tannery wastewater presents a great challenge to experts in the field of wastewater management(Di Laconi *et al.,* 2002;Schrank *et al*., 2009).Tannery wastewater generates obnoxious gases thereby contaminates the air (Schilling *et al.,* 2012; Shakir *et al*., 2012) and possible poisoning from toxic gases(Raman *et al.,*2012; Prier *et al*., 1984 also affect microorganisms in soils thereby reducing fertility of agricultural land (Jochimsen and Jekel, 1997 ;Szpyrkowicz *et al.,* 2001;2005), hence can affects the ecological system negatively [28]; [10]. These constitute serious environmental problems (Oral *et al.,* 2007:Szpyrkowicz et al.,2005). Pollutants range from organic matters to trace metals and ions well above tolerable limits (Szpyrkowicz et al.,2005). Discharge of this effluent into open land may eventually percolate and affect the underground water or could be washed to water bodies, damaging the quality of the receiving streams(Charles, 2010; COD, 2015). These pollutants may be toxic to food chain organism and aquatic life (Raman et al., 2012).

Treatment of Wastewater and its Effects

Different methods of wastewater treatment have been conducted; each is associated with its challenges and prospects (Orhon, et al., 1998; Gasparikova et al., 2005;Amokrane et al.,1997). Some methodologies may not be economically feasible because of high cost or expertise required for implementing and sustaining the operation of such processes (Orhon, et al., 1998;Schrank et al., 2009). 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 (Augusto and Maia 1998).

There has been recent growing interest regarding anaerobic treatment of tannery wastewater due to the several advantages over other methods of tannery effluent treatment. The method is gaining popularity over the aerobic method as it is cost effective, require less energy and less maintenance cost (Banu and Kaliappar, 2007;Dixit et al., 2014):Durai and Rajassman 2011); (http://www.sciencepub.net/nature). In this method anaerobes use other sources of oxidation to abstract energy for their respiration. Organic materials in the tannery wastewater may be used as a source of carbon donating electrons to the Sulphur which is then reduced (Di Laconi et al., 2002; Durai and Rajassman, 2011). By this oxidation-reduction, the microorganisms' abstracts energy, consequently the organic materials are depleted and the pollutants in the wastewater gradually gets reduce by degradation(Mrowiec and Suschka, 2006). This method of tannery wastewater treatment is however associated with some drawbacks which include: High protein component in the wastewater which affects the selection of biomass, slow kinetics or hydrolysis, inhibits granular sludge formation (Lofrano et al., 2013; Pierce et al.,1984)

Anaerobic wastewater treatment has an advantage of low sludge formation, removal of higher organic loading and high pathogenic organisms (Joanna et al., 2003), methane gas production and low energy consumption (Weimann et al., 1998; Vijayaraghvan and Murthy, 1997). However, anaerobic treatment systems have been reported to have sustainable technology for initial wastewater treatment because of its low cost of operation and easy maintenance, small land requirement(Joanna et al., 2003; Kumar et al., 2002; Banu and Kaliappar 2007).).

Two basic methods are employed in anaerobic wastewater treatment processes. These are Psychrophilic and Mesophilic processes. The psychrophilic anaerobic treatment process is usually at lower temperature (<20^oC) (Zupanie et al., 2007;Elmitwalli et al., 1999) while Mesophilic treatment process is carried out at temperature of about 35^oC – 37^oC (Nykova et al., 2002;Parawira et al., 2005). This means that the temperature of thewastewater to be treated using either psychrophilic or mesophilic process is important and needs to be adjusted.Under psychrophilic condition, chemical and biochemical reactions have been reported to proceed much slower than mesophiliccondition (Durai and Rajassman, 2011; Zupanie et al,. 2007)

MATERIALS AND METHODS

Isolation of Anaerobic Bacteria from Aged Sulphide-Lime Wastewater

The isolation of five (5) different anaerobic bacteria was carried out according to standards procedures (Pepper and Gerba, 2004). An agar was prepared in five Petri dishes and kept overnight in a refrigerator and sterilized the next day. The agar was inoculated with sample of aged sulphide-lime unhairing liquor taken at different state as follows: supernatant sulphide-lime unhairing liquor- T_I and T_{II}; stirred sulphide –lime unhairing liquor-T_{III} and T_{IV}; while; aged re-liming liquor-T_V. The five inoculated Petri dishes were placed in an air tight anaerobic gas jar and incubatedanaerobically at 37^oC for 48 hours

Treatment of Sulphide-lime Unhairing Tannery Wastewater

The pH of filtered tannery sulphide-limed wastewater was adjusted to 7.0 using diluted HCl (1:1) and was heated to 70^0C with the temperature maintained for fifteen minutes, covered and left overnight. This was repeated for three consecutive days. Then 500ml was reserved as control sample and the remaining distributed into 120ml plastic bottles each containing 100ml of filtered sulphide-lime unhairing wastewater. A 0.5ml of sodium alginate was added and shaken to prevent the sampled wastewater from settling. Triplicate Samples wastewater were inoculated with each isolate, according to Standard procedures and incubated anaerobically at room temperature, 27^0C and 37^0C. Then Pollutants govern by COD and BOD were determined at 5, 7 and 9 days, to estimate the reduction of each parameter. All analysis was conducted according to standard procedures as reported by USEPA, 2012, for wastewater assessment and monitoring.

Determination of Biochemical Oxygen Demand (BOD)

The azide modification of the Winkler method was adopted as reported byUSEPA, 2012. Sample(0.5ml) of wastewater was measured into a 300mlBOD bottle. Then 2.0ml of manganese sulphate solution, 2.0ml of alkaline iodide-azide reagent was 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 to allow the precipitates to settled, leaving a clear supernatant above the floc, the bottles were shaken again and allowed to stand for a minute. Then 2.0ml of concentrated H_2SO_4 was carefully added into the mixture and inverted 2-3 times and allow standing for the precipitate to settle again. Then, 200ml of supernatant was measured into 500ml conical flask and 1.0ml of starch indicator was added. Each sample was titrated with 0.025M of $Na_2S_2O_3.5H_2O$ to a pale straw color.

The dissolved oxygen in each wastewater sample was calculated on the basis that; 1.0 ml of 0.025M sodium thiosulphate titrant is equivalent to 0.2mg DO. Thus each milliter of sodium thiosulphate titrant used is equivalent to 1mg/L DO. The same procedure was repeated after five days incubation in the dark at 20±2^0C for BOD_5. Then BOD calculated from the difference between initial and final DO using the expression below;

$$BOD_{5=}\ as\ \left(\frac{mg}{L}\right) D_{O-}D_5/\mathrm{p}$$

Where,

D = is the DO of the diluted sample solution (mg/l)

P = is the decimal dilution factor

D_5 = is the DO of diluted sample after 5-day incubation (mg/l)

Determination of Chemical Oxygen Demand (COD) - Open Reflux Method (USEPA, 2012).

Following the procedure,20ml of (0.5:100)diluted Sample wastewater wastaken and mixed with chromic and sulphuric acids and the mixture was shaken. Then 0.4g of $HgSO_4$ mixed with 10 ml standard $K_2Cr_20_7$ solution was added and a few pieces of anti-bump granules also into the flask and oxidized by boiling the mixture. The flask was then attached to the reflux condenser and 30ml of concentrated H_2SO_4 containing $AgSO_4$ was slowly added into the flask and mixed thoroughly. The mixture was refluxed for one hour, cooled and the condenser rinsed with 25ml of distilled water. Then diluted to 100ml with distilled water, and allow to cool. Three drops of ferroin indicator was added and the mixture titrated with standard ferrous ammonium sulphate (Fe $(NH_4)_2SO_4$) until a sharp color change was observed from blue-green to reddish brown. A blank was prepared and run with all the reagents except the sample.

The chemical oxygen demand in each sample was calculated using the expression:

$$\mathrm{COD\ as}\ (\frac{\mathrm{mgO_2}}{\mathrm{L}}) = \frac{(\mathrm{A-B})\mathrm{MX800}}{\mathrm{ml\ of\ sample\ taken}}$$

Where:

A = Volume of Fe $(NH_4)_2SO4$ used for blank
B = Volume of Fe $(NH_4)_2SO4$ used for sample
M = Molality of $Fe(NH_4)_2SO_4$ used in titration

RESULTS AND DISCUSSION

Table 1.1 - 1.3 shows statistical results of the effect of time, temperature and individual isolates on the treatment of the wastewater and the various significant levels attained. From Table 1.1 it is observed that BOD parameter is not significantly different through the treatment temperatures while, COD is significant between day five and nine. For the different isolates both parameters response was significantly different from the control samples, Tables 1.1–1.3

The anaerobic treatment of sulphide-lime unhairing wastewater with 0.5 McF at 25 °C for 5 – 9 days is shown in Figure 1.1 – 1. 3. The results indicate COD having percentage reduction of about 40 % for all the isolates for room and 27°C temperature but, asit is raised to 37°C for the same period, further reduction of COD was observed while, BOD remained below 20% Figure 1 and Figure 2 showed COD having the highest percentage reduction of about 50 %, while BOD_5 was less than 40 %, except for$T_{IV}$and T_I at 27°C and 37°C respectivelyafter treating thewastewater with 0.5 McF at 37°C for a period of 7 days. However,$T_{III}$was an exception which indicated a reduction of 60% as the highest value.

Table 1.1: Mean Reduction of the Parameters at Different Temperatures

Temp.	N	COD (mg/L)	BOD (mg/L)
Room Tempt	66	17014.4±5243.06[a]	1064. 5±109.80[a]
27°C	66	15395.6±4907.63[b]	891.7 ±249.06[a]
37°C	66	14185.2±5405.02[c]	866.2 ±815.12[a]
Total	198	15531.7±5292.11	940.8 ±800.95

Notes: Means with the same letter are not significantly different at 0.05

Table 1.2.: Mean Reduction of Parameters at Different Period of Treatment

PERIOD	N	COD (mg/L)	BOD (mg/L)
DAY 5	66	17871.2±4003.14[a]	1092.7 ±168.86[a]
DAY 7	66	15845.5±5098.32[b]	1135.0 ±129.79[a]
DAY9	66	12878.5±5482.48[b]	594.8 ±242.25[b]
Total	198	15531.7±5292.11	940.8 ±800.95

Notes: Means with the same letter are not significantly different at 0.05

The result obtained on the percentage reduction of pollutants Figure 3 after day 9 treatment periods of wastewater with the five isolates using 0.5 McF at both 25°C and 27°C indicated significantly reduction of COD and BOD_5 to about 50%.But as the temperature increases to 37°C BOD was observed to reduce further than 60% while COD remained less than 60% except for T_{IV} isolate at 27°C and 37°C after 9 days' treatment period

Table 1.3: Mean Reduction of the Parameters by Different Isolates after treatment

TREATMENT	N	COD Mean ± SD	BOD Mean ±SD
CONTROL	18	28833.3±923.55[a]	1280.0 ±24.49[a]
TI	36	14526.9±3452.10[bc]	836.9 ±292.31[b]
TII	36	14283.1±3731.01[c]	803.1 ±254.26[b]
TIII	36	13761.1±2970.51[cd]	837.8 ±256.74[b]
TIV	36	13072.8±2948.92[d]	812.4 ±259.67[b]
TV	36	15363.9±3279.52[b]	1244.4 ±176.83[a]
Total	198	15531.7±5292.11	940.8±800.95

Notes: Means with the same letter are not significantly different at 0.05

Figure 4 showed similar trend as observed in Figures 1 – 3, the effects of 1.0 McF standard of isolates for 5 - 9days' treatmentindicated different levels of significance with COD having higher reductive response while BOD remained low for day 5 – 7 days of treatment as shown in Figures 4 and 5.The effects of anaerobes on the treatment of wastewater with 1.0 McF at all temperatures shows improvement in the reduction of BOD parameter, though the COD remained higher in the reduction by all the isolates for 7 days as shown in Figure 5. With increase in the treatment temperatures and period to 9 days the two pollution parameters further reduced significantly above 60% as observed in Figure 6.

The Effects of Anaerobic Treatment of the unhairing lime Wastewater

The five isolates used for the treatment of sulphide-lime unhairing and liming wastewater with a blank sample wastewater as control (CTL), COD, BOD_5 parameters were determined before and after anaerobic treatment of sulphide-lime unhairing/liming wastewater.

The data collected wasanalyzed with the general linear model (GLM) to assess the level of reduction of pollutants by the isolates at different temperatures, periods of treatment and two McFarland standards values. Means obtained from the ANOVA, along the post hoc test on the means using Duncan Multiple Range Test (DMRT) Table 1.1, 1.2 and 1.3. Classes of effectiveness in pollutants reduction are indicated alphabetically in descending order. The computation took into considerations all temperatures, different period of treatment and McFarland standards.

For BOD_5, the differences in reduction observed throughout the treatment temperatures for each period was not significant ($P > 0.05$) (Table 1.3). The COD results showed some dependence on temperature as indicated by a significant difference ($P < 0.05$).This suggests that the degradation which led to reduction of COD by the anaerobes is temperature dependent (Zupanie et al., 2007; Elmitwalli et al.,1999). For COD, the effect of treatment period for day 5 was significantly different from day 7 and 9. Considering, BOD_5 parameter, it was observed that its reduction was significantly different only between day 7 and 9 Table 1.2 ($P < 0.05$). The highest percentage reduction obtained was for Day 9 and least for Day 5.This suggest that treatment period is an important condition to be considered in anaerobic treatment of sulphide-lime wastewater.

For individual isolates the reduction of COD parameter exhibited significance difference between the control and the isolates ($P < 0.05$), Table 1.3. The effects of the isolates on COD parameter showed significance difference between the isolates. This suggest the individualistic characteristics and efficacy of the different isolates and their treatment action on the wastewater. For BOD_5, the reduction on the parameter showed significant difference between the CTL sample and other isolates treated wastewater ($P < 0.05$), but non exits between isolates except isolate T_V (Table 1.3). The trend suggests that the use of these anaerobic isolates for the treatment of sulphide-unhairing liming wastewater has high prospects.

Effects of 0.5 McF on the Treatment of Sulphide-lime Wastewater

The treatment of sulphide-lime unhairing wastewater with 0.5 McF at 25°C for 5 days achieved only a minimal percentage reduction of BOD but was significant with the COD parameter. However, at day 7, the isolates showed higher reductive effects as period of treatment increases. Consequently, at 25°C with 0.5McF and 9 day treatment period, T_{II}, T_{III} had higher reduction values of 58.5 % and T_{IV} having 67.94 %. This trend suggests that temperature had influenced the activity of the anaerobes, similar to a report by Tilley *et al.*, 2013, Figure 1 At, the end of day 9 treatment period, the maximum reduction of pollutants due to BOD_5 was 71.43 % by isolate T_V at 27 °C, Figure 3. Considering the effectiveness of individual isolates, it was observed that at day 9, the highest percentage reduction of BOD_5 was recorded by isolate T_{IV} at 67.94 %; 65.0 % and 69.84 % at room temperatures, 27°C and 37°C respectively with 0.5 McF, implying that the isolates are likely to be mesophilic (35°C – 37°C),(Mrowiec and Suschka, 2006). Further reduction of COD was recorded when treatment temperature and time was increase to 37°C Such that after day 9 treatment periods, isolate T_{IV} indicated a reduction of 62.5 % and 79.29 %, at 27°C and 37°C respectively as shown in Figure 3

Effects of 1.0 McF on the Treatment of Sulphide-lime Unhairing Wastewater

The increase of 0.5 – 1.0 McF did not showed much influence on percentage reduction of BOD_5. However, further reduction in BOD_5 was observed when treatment period was extended, Figures 4 – 6. By day 9, its percentage reduction was observed to increase for most isolates particularly $T_{II,}$ T_{IV} and T_V (Figures 4). At 1.0 McF, isolate T_V had 93 % as the highest percentage reduction in BOD_5 by day $7_,$ even though it was abrupt, it was observed to have the greatest potentials for the reduction of pollutants measured by BOD_5.

Similarly,with 1.0 McF standard, Isolates T_{IV} also reduced COD in the wastewater by 67.07 % at 27 °C while T_V by 69.91 % at 37 °C. The trends suggest that temperature and period of treatment influences BOD reduction due to the anaerobic activities already observed. For the same period and increasing the size of the inoculum to 1.0 McF, 68.33 % reduction of COD was observed at 27°C, while 67.07 % reduction of this parameter was recorded for isolate T_{III} at, 27 °C. The maximum reduction of COD with 1.0 McF at 37 °C for day 9 treatment period was 69.91 % triggered by isolate T_V. 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 (Pfenning and Biebel,1986).

RECOMMENDATION

Isolates T_{IV} and T_V stand out in the reduction of BOD from the wastewater while isolates T_{III} and T_{IV} gave very promising results for the reduction of COD parameter. The anaerobic treatment of sulphide-unhairing limed wastewater was observed to reduce pollutants determined by COD significantly irrespective of time and McF standard, while that of BOD was gradual and steady These anaerobes may be employ in the treatment of this type of wastewater and most probablyoptimization method might yieldbetter results.

CONCLUSION

Although the anaerobic treatment still left the pollutants very high and well and above the recommended acceptable limit for wastewater discharged by WHO, UNEPA and NESREA. The study was to estimate the level of percentage reduction of pollutants in the wastewater.

Degradation ability of the microorganisms was determined using BOD and COD, which are principal parameters often considered in determining the quality of any wastewater for reuse, recycle or for irrigation purposes. The treatment conditions of the wastewater were at room temperature (22 °C) , 27°C and 37°C for 5,7 and 9 days with different McFarland standards of 0.5 and 1.0 McF.

Considering the ability of individual isolates, it was observed that isolates T_{IV} and T_V stand out in the reduction of BOD from the wastewater while isolates T_{III} and T_{IV} gave very promising results for the reduction of COD parameter. The anaerobic treatment of sulphide-unhairing limed wastewater was observed to reduce pollutants determined by COD significantly irrespective of time and McF standard, while that of BOD was gradual and steady. Meanwhile both parameters were influenced by temperatures and period of treatment.

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Appendix 1

Figures 1 – 6 showing various responses of the effects of different conditions of treatment on the sulphide-lime wastewater

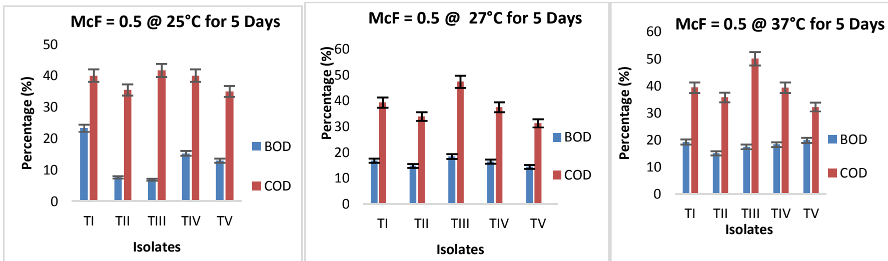

Figure 1: Effect of 0.5 MFc Standard on the treatment of the wastewater at Different Temperatures for 5 Days

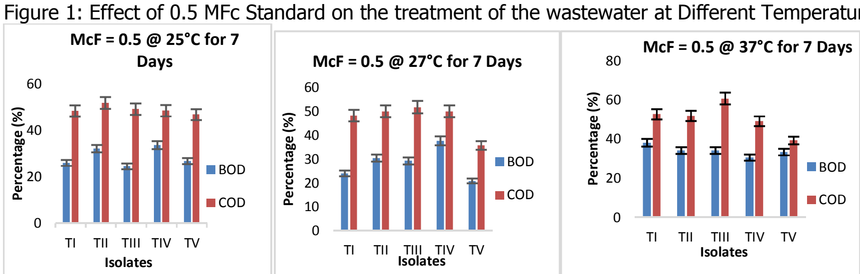

Figure 2: Effect of 0.5 MFc Standard on the treatment of the wastewater at Different Temperatures for 7 Days

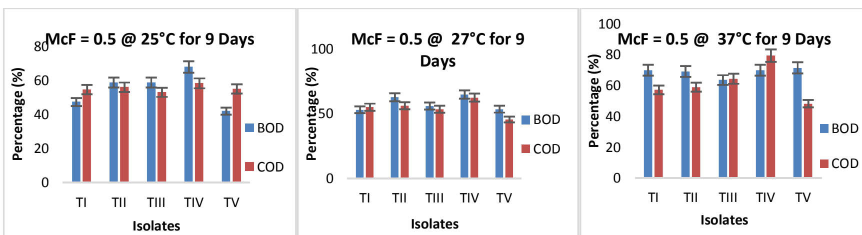

Figure 3: Effect of 0.5 MFc Standard on the treatment of the wastewater at Different Temperatures for 9 Days

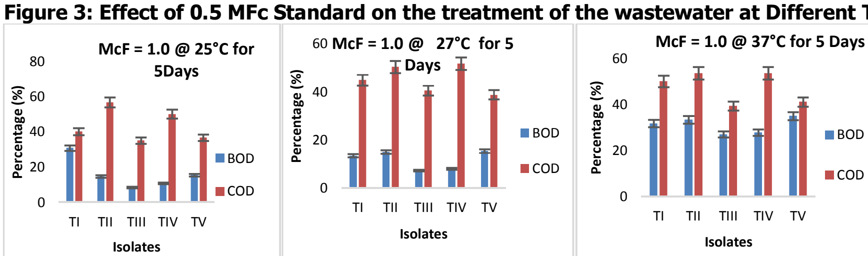

Figure 4: Effect of 1.0 MFc Standard on the treatment of the wastewater at Different Temperatures for 5 Days

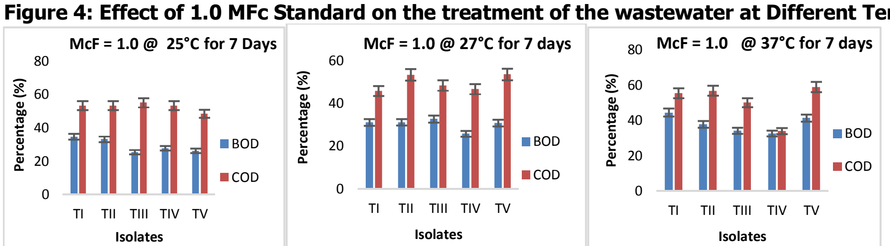

Figure 5: Effect of 1.0 MFc Standard on the treatment of the wastewater at Different Temperatures for 7 Days

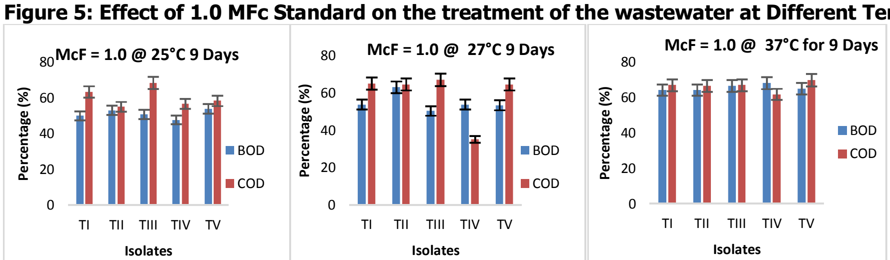

Figure 6: Effect of 1.0 MFc Standard on the treatment of the wastewater at Different Temperatures for 9 Days