



EFFECT OF ANAEROBIC TREATMENT ON SULPHIDE CONCENTRATION, TSS, TDS AND ELECTRICAL CONDUCTIVITY PARAMETERS OF SULPHIDE-LIME UNHAIRING WASTEWATER

*¹Tanko, S.F., ²Ajibola, V.O., ²Agbaji, E.A., ²Mukailu, A.S., ¹Putshaka, J. D¹. and Amana, B.S.

¹Nigeria Institute of Leather and Science Technology, P.M.B., 1034 (NILEST) Zaria, Nigeria ²Department of Chemistry, Ahmadu Bello University (ABU) Zaria, Nigeria.

*Correspondence author: Solomontanko0@gmail.com

ABSTRACT

Five microorganisms were isolated from aged sulphide-lime tannery wastewater following conventional method. The same was inoculated into similar wastewater collected after the production of upper leathers from Sokoto red goatskins, Nigeria. The effects of anaerobic treatment of the tannery effluent resulted to the reduction of pollutants determined by Sulphide and TSS and reducing sludge formation expected from sulphide-lime effluent. However, TDS which was observed as being proportional to electrical conductivity increase with respect to time, temperature and McF standard. At day 9 with 1.0 McF, isolate T_{II} effected an increase in TDS by 51.09 % at 25°C and 64.03 % at 27°C, while isolate T_I had an increase in TDS to 81.67 % and T_V to 80.12 % at 37°C as the highest percentage recorded. Isolate T_I was observed to increase electrical conductivity by 5.9 % at 25°C with 0.5 McF, 45.5 % at 27°C and isolate T_{III} by 52.83 % at 37°C for the same period of treatment. At day 7 with increase temperatures, the same isolate had 108.22% increase in electrical conductivity at 27°C and 104.85 % at 37°C. Although increase of electrical conductivity of the treated wastewater is expected, this abrupt increase is abnormal, and may be due to complex nature of tannery wastewater. The maximum percentage reduction of sulphide was 25.35 % and 20.42 % by day 5 with 1.0 McF at 25°C, for isolates T_{III} and T_V respectively. This is most probable because the anaerobes that induced the oxidation of carbon content of the wastewater while abstracting energy for their respiration. This causes Sulphur to get reduced to sulphide bringing about an increase of the parameter at the beginning of the treatment later decline, while depleting the carbon substances in the wastewater.

Keywords: wastewater, Anaerobic, Microorganisms, Treatment, Oxidation

INTRODUCTION

Sulphide-lime Unhairing wastewater is known for high values of pollutants often determined by parameters such as COD, BOD as well as Sulphide Concentration, TDS, TSS and electrical conductivity and so on. The effluent is complex and said to constitute 65 – 70% pollutants in a combined tannery wastewater, thus its treatment is inevitable. The low biodegradability tendencies of tannery wastewater present a great challenge to professionals in the field of wastewater management (Di Laconi *et al.*, 2002). Tannery wastewater causes obnoxious gases thereby contaminates the air (Schilling *et al.*, 2012; Shakir *et al.*, 2012) and possible poisoning from toxic gases (Raman *et al.*, 2012), this can also affect the ecological system negatively (Kumar, 2008; Siqueira *et al.*, 2011). Disposal of this type of effluents to open land without proper treatment, eventually percolate and affect the underground water or washed to water bodies, destroying aquatic life of the receiving streams (Charles, 2010; COD, 2015). These pollutants may be toxic to food chain organism of an ecological systems (Raman *et al.*, 2012). Fertility of soils for agricultural activities and the eventual yield may be affected, besides bioaccumulation of some heavy metals which may pose as health threats.

Several methods of wastewater treatment exist; precipitation, coagulation, flocculation and so on, but each method is associated with one or more challenges (Lefebvre *et al.*, 2006a and 2006b). However, anaerobic treatment method is becoming

more acceptable, as it does not use chemical reagents and its eventual generation of biogas from the process is used as an energy source to reduce the cost of the process. The method is cost effective, require less energy and less maintenance cost (Dixit *et al.*, 2014; Durai and Rajassman, 2011; Kumar *et al.*, 2002).

In this method anaerobes induced oxidation and abstract energy for their respiration. Organic materials in the tannery wastewater is used as a source of carbon donating electrons to the Sulphur which is then reduced to sulphide (Di Laconi *et al.*, 2002; Durai, *et al.*, 2011). By this oxidation-reduction, organic materials are depleted and the pollutants in the wastewater gradually gets reduce by degradation (Lofrano *et al.*, 2013). High protein component in the wastewater which affects the selection of biomass, causing slow kinetics or hydrolysis and inhibits granular sludge formation. (Joanna *et al.*, 2003). The process is either psychrophilic that is at lower temperature (<20°C) or mesophilic - 35°C – 37°C (Parawira *et al.*, 2005).

MATERIALS AND METHODS

Isolation of Anaerobic Bacteria from Aged Sulphide-Lime Wastewater

Five (5) anaerobes were isolated according to standards procedure as described by (Pepper and Gerba, 2004). Nutrient 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 effluent taken at different state: supernatant sulphide-lime unhairing effluent- T_I and T_{II}; stirred sulphide-lime unhairing effluent, 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 incubated anaerobically at 37°C for 48 hours.

Treatment of Sulphide-lime Unhairing Wastewater

The filtered tannery sulphide-limed wastewater was adjusted topH 7.0 with diluted HCl (1:1) and heated to 70°C and maintained for fifteen minutes, covered and left overnight. This was repeated for three consecutive days. Then 500ml was reserved as CTL sample and the remaining distributed into 120ml plastic bottles each containing 100ml of the filtered wastewater. A 0.5ml of sodium alginate was added and gently shaken to prevent the sampled wastewater from settling. Triplicate samples were inoculated with each isolate and incubated anaerobically at room

temperature, 27°C and 37°C. Then Pollutants determined by sulphide concentration, TDS, TSS and electrical conductivity at 5, 7 and 9 days, to estimate the decline of each parameter. All analysis of the parameters was conducted according to standard procedures as described by (USEPA, 2012) for wastewater assessment and monitoring.

Determination of Sulphide (S²⁻) – Iodometric Method

The sample wastewater was diluted with distilled water in the ratio of 1:20 and thoroughly mixed in a 500 cm³ beaker and 200 cm³ of the mixture was withdrawn into a 500 cm³ conical flask. Then 2.0 cm³ of standard iodine solution together with 2.0 cm³ 6.0 M HCl was gently added after shaken. 1.0 cm³ Starch indicator was added and thoroughly mixed by shaking and titrated with sodium thiosulphate (0.025 M) until the solution turned light brown as reported in the procedure by Arnold et al., 1992. Then S²⁻ concentration in each sample was calculated using the expression:

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

A– Volume of standard iodine solution

B – Molarity of standard iodine solution

C – Volume standard Na₂S₂O₇ titrant

D – Molarity of standard Na₂S₂O₇ titrant

Determination of Total Suspended Solids - TSS

Sample wastewater equivalent to 20 cm³ was filtered through a pre-weighed filter paper (What man No.1) after which the filter paper was dried at 103 ±2°C to

$$\text{TSS } \left(\frac{\text{mg}}{\text{L}} \right) = \frac{W_2 - W_1}{\text{Volume of Sample taken}} \times 1000$$

Where:

W₁ – weight of filter paper

W₂ – weight of filter paper + residue

Determination of Total Dissolved Solids (TDS)

Wastewater (20 cm³) was filtered through filter paper (What man No.1) into a pre-weighed beaker, and evaporated to dryness on a hot plate for one hour at

$$\text{TDS } \left(\frac{\text{mg}}{\text{L}} \right) = \frac{W_2 - W_1}{\text{Volume of Sample taken}} \times 1000$$

Where:

W1 – weight of Beaker

W2 – weight of Beaker + residue

Determination of Electrical Conductivity

Electrical conductivity of wastewater was determined by direct measurement of the conductivity of the wastewater using Electrolytic conductivity measuring set, (Model MC-1. Mark V) made in England. The cell

a constant weight. The increase in weight of the filter paper was noted and total suspended solid was calculated using the formula

103 ±2°C. The beaker was then transferred to a desiccator and allowed to cool, then weighed. The total dissolved solid was calculated using the formula:

was gently filled with test sample ensuring there were no air bubbles adhering to the electrode. The measurement for each sample was conducted at room temperature (23°C).

RESULTS

Table 1: Mean Reduction of the Parameters at Different Temperatures

Temp.	N	SULPHIDE (mg/L)	TDS (mg/L)	TSS (mg/L)	E. COND. ($\mu\text{S}/\text{cm}^3$)
Room Tempt	66	633.6±73.69 ^a	25788.2±4770.22 ^b	9969.2±1653.92 ^b	15981.8±13416.77 ^a
27°C	66	612.2±47.27 ^b	26785.3±4337.11 ^{ab}	10548.0±3002.52 ^{ab}	19382.3±7338.41 ^b
37°C	66	605.1±29.24 ^b	27500.0±5540.66 ^a	11078.2±5116.01 ^a	23388.7±8525.617 ^b
Total	198	617.0±54.39	26691.2±4933.45	10531.8±3566.40	19584.3±10505.04

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

Table 2: Mean Reduction of Parameters at Different Period of Treatment

PERIOD	N	SULPHIDE (mg/L)	TDS (mg/L)	TSS (mg/L)	E. COND. ($\mu\text{S}/\text{cm}^3$)
DAY 5	66	626.3±53.29 ^a	22823.2±2702.87 ^c	13614.3±2825.08 ^a	14852.7±13035.20 ^a
DAY 7	66	627.4±39.05 ^a	27223.2±4439.10 ^b	10085.5±2351.36 ^b	20180.8±6566.21 ^b
DAY9	66	597.2±63.27 ^b	30027.1±4469.47 ^a	7895.6±2851.68 ^c	23719.3±8998.71 ^c
Total	198	617.0±54.39	26691.2±4933.45	10531.8±3566.40	19584.3±10505.04

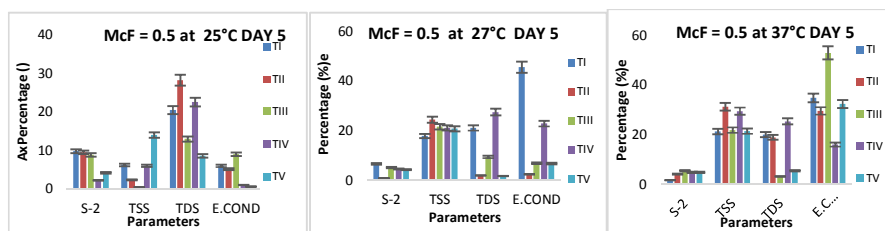
Notes: Means with the same letter are not significantly different at 0.05

Table 3.0: Mean Reduction of the Parameters by Different Isolates after treatment

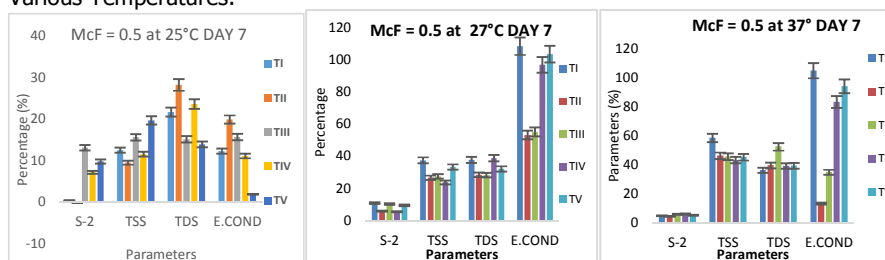
TREATMENT	N	Sulphide Mean±SD	TDS Mean±SD	TSS Mean±SD	ELECT. COND Mean±SD
CONTROL	18	580.7±11.94 ^b	20755.0±191.47 ^c	15158.3±2478.57 ^a	12104.2±2966.30 ^a
TI	36	626.7±63.98 ^a	27694.2±4097.35 ^{ab}	9504.4±3474.55 ^b	22859.1±185003
TII	36	615.2±62.62 ^a	27683.0±4810.60 ^{ab}	10641.3±3319.96 ^b	18938.3±7321.36 ^b
TIII	36	625.3±57.42 ^a	26049.7±4013.22 ^b	10464.4±2933.36 ^b	19277.7±6924.56 ^b
TIV	36	618.1±46.52 ^a	28638.3±3130.42 ^a	10045.3±3585.40 ^b	20105.4±7549.76 ^b
TV	36	617.6±47.48 ^a	26358.6±6821.58 ^b	9690.3±3312.12 ^b	20480.9±8637.95 ^b
Total	198	617.0±54.39	26691.2±4933.45	10531.8±3566.40	19584.3±105054

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

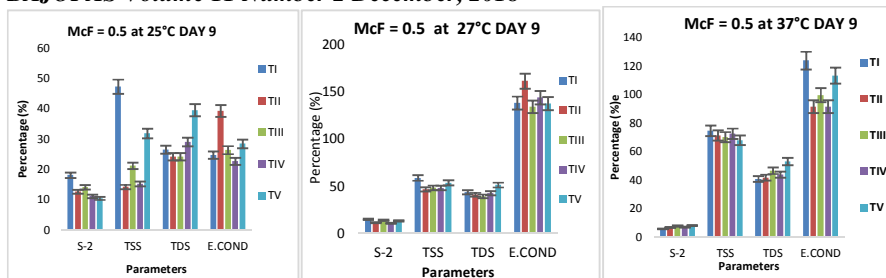
Effects of Anaerobic Treatment on Sulphide Concentration, TSS, TDS and Electrical Conductivity of Sulphide-Lime Unhairing Wastewater at Different Conditions of Treatment.



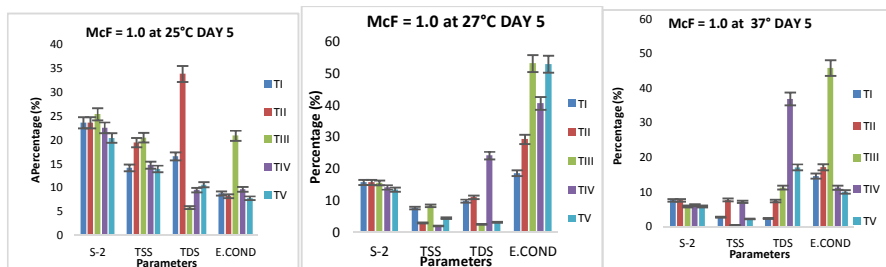
Figures 1: Effects of Anaerobic Treatment of the Wastewater for Five Days with 0.5 McF Standard at Various Temperatures.



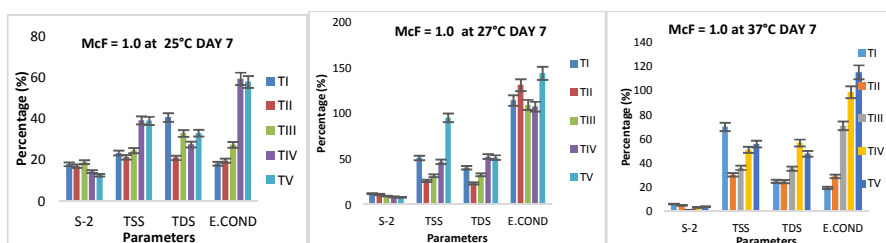
Figures 2: Effects of Anaerobic Treatment of the Wastewater for Seven Days with 0.5 McF Standard at Various Temperatures



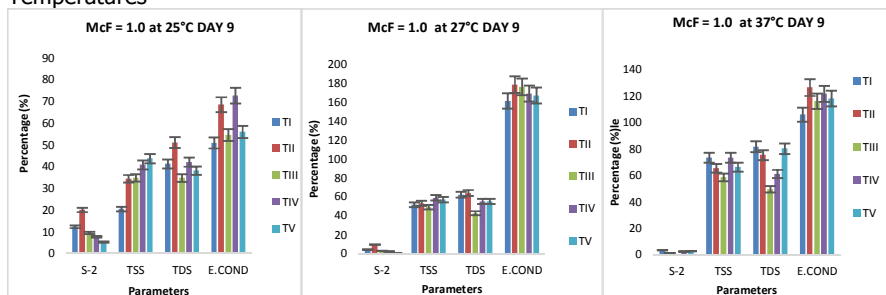
Figures 3: Effects of Anaerobic Treatment of the Wastewater for Nine Days with 0.5 McF Standard at Various Temperatures



Figures 4: Effects of Anaerobic Treatment of the Wastewater for Five Days with 1.0 McF Standard at Various Temperatures



Figures 5: Effects of Anaerobic Treatment of the Wastewater for Seven Days with 1.0 McF Standard at Various Temperatures



Figures 6: Effects of Anaerobic Treatment of the Wastewater for Nine Days with 1.0 McF Standard at Various Temperatures

DISCUSSION

Anaerobic treatment of sulphide-lime unhairing wastewater was observed to show general reduction of all the parameters determined after the treatment process. From Table 1 the mean reduction of parameters at different temperatures showed a general reduction of sulphide concentration as temperature increases. This is similar to the effects of time on the treatment process observed in Table 2 as the treatment period increases. The individual effects of the isolates showed general increase of the sulphide concentration from the control, which is in agreement with the results expressed in the Figures 1 – 6 below. This increase of the sulphide concentration is as a result of oxidation of carbon source from the

wastewater leading to the reduction of Sulphur to sulphide (Lofrano *et al.*, 2013) thereby increasing its concentration at the beginning of treatment. Anaerobic treatment also affected the TSS positively as it shows reduction of the parameters from the control as treatment period increases, Table 3. However, TDS and electrical conductivity were observed to be proportional, as TDS and electrical conductivity were observed to increase instead of reduction. This is as results of degradation and freeing of the ions from the matrices of the wastewater hence the increased electrical conductivity. This makes it easy for the ions to be precipitated or immobilization, hence the possible removal of the trace metals from the wastewater.

The effects of anaerobic treatment with 0.5 McF Standard of sulphide-lime unhairing wastewater indicated poor percentage reduction of TSS at 25°C for day 5 response was low. However, with increase in the treatment temperature, TSS reduction was raised to 31.22 %, 29.42 % by T_{II} and T_{IV} isolates in that order, Figure 1.0. Further percentage reduction was observed by isolates T_I to 37.36 % and 58.43 % for day 7 with 0.5 McF at 27°C and 37°C respectively. Similarly at day 9, the same T_I caused further reduction of TSS to 47.13 % at 25°C; 55.48 % at 27°C and 74.44 % at 37°C. This trend confirms the effects of increasing time and temperature on the degradation process of the anaerobic microorganisms on the sulphide-lime wastewater.

Generally higher reduction of sulphide concentration was observed in the treatment of unhairing wastewater particularly at the beginning, then declined steadily as observed in Figures 2 and 3 then subsequently declined further as the period of treatment and temperatures increases Figures 4, 5 and 6. The most effective isolate identified in the reduction of this parameter with 0.5 McF at 25°C; was T_I which recorded a reduction of only 9.75 %, 10.76 % and 18.02%, Figures 1, 2, and 3 respectively. Other isolates also displayed their abilities at various levels.

From Figure 1, it was observed that there was a poor response of TDS after 5 days treatment with 0.5 McF at 25°C. However, isolates T_I, T_{IV} were observed to cause increase of TDS parameter by 21 % and 27.42 % respectively, with further increase of temperature to 37°C the same isolates were observed to cause further reduction of the parameter Figure 2.0. Other isolates showed very similar increasing trend of the parameter, for example, at 0.5McF at 25°C, day 5, T_I indicate 20.44 % and T_{II} 28.22 % of TDS. With similar conditions of treatment T_I and T_{IV} increased TDS to 37.67 % and 38.76 % at 27°C correspondingly, at 37°C T_I and T_{III} raised the TDS to 36.19 % and 52.38 % respectively. This observation on the effects of temperatures further supports the reports that these anaerobes are possibly mesophylls, (Tilley *et al.*, 2013). Figures 5 and 6.

Electrical conductivity of the anaerobic treated wastewater was observed to increase with all isolates at different time and temperatures. Isolate T_I was observed to increase electrical conductivity by 5.9 % at 25°C with 0.5 McF, 45.5 % at 27°C and isolate T_{III} by 52.83 % at 37°C for the same period of treatment Figure 1. At day 7 with increase temperatures, the same isolate had 108.22% increases in electrical conductivity at 27°C and 104.85 % at 37°C. Although increase of electrical conductivity of the treated wastewater is expected, this abrupt increase is abnormal, and may be due to complex nature of tannery wastewater (Wiegant *et al.*, 1999; Zang *et al.*, 2002; Bhatia *et al.*, 2006; Abohassan *et al.*, 2008). Another reason could be due to the high level of freed ions which the instrument could not respond effectively (Arnold *et al.*, 1992) as previously reported. However, extreme cases of very low conductivity were also observed for isolates T_{IV} and T_V, Figure 1; isolate T_{II}, T_{III} and T_V, Figure 2. Considering the electrical conductivity response to the individual isolates, it is difficult to pick a leading

microbe. However; isolates T_{II} and T_{III} seem to show higher ability in the degradation and dissolution of the wastewater.

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

Considering TSS, increase temperature and McF standard from 0.5 to 1.0 McF, percentage reduction of TSS by isolates T_{III} and T_{II} was as low as 8.29 % and 7.74 % respectively (Figure 1). This may be attributed to the presence of some components of the wastewater that were possibly difficult to degrade. Another possible reason could be increased size of inoculum lacking sufficient nutrients and the microbes began a process of mutation. However, at day 7, percentage reduction of TSS increased to 39.14 % at 25°C with isolate T_{IV} and 38.93 % at 27°C with 1.0 McF. Maximum reduction of TSS up to 95.23 % was achieved by T_V isolate followed by T_I recording 51.32 % reduction. The observed percentage reduction in TSS was similar to reports by other researchers such as (Nykova *et al.*, 2002; Muler *et al.*, 2002; Varsha and Apurba, 2008). From Figures 4 – 6, it was observed that increase in temperature favoured the activities of these microorganisms, supporting the anaerobes as possibly mesophilic (35°C – 37°C) (Tilley *et al.*, 2014). T_I and T_{IV} isolates were consistent in the reduction of TSS in the wastewater. Although isolate T_V with 95.23 % of TSS reduction, was abrupt at day 7 with 1.0 McF standard, it is indicative that the effects of these anaerobes on the wastewater may be linked to their ability to oxidize sugars, converting large molecules of organic substances to smaller particles size or even to gaseous forms i.e. CH₄, CO₂, H₂S and so on (Nykova *et al.*, 2002).

The evolution of Sulphide (S²⁻) observed from the anaerobic treatment of sulphide-lime unhairing wastewater may likely posed negative effects, poses serious health threats similar to reports by Lofrano *et al.*, 2013; Varsha and Apurba (2008). From Figures 1, 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 percentage reduction of 9.75 % at 25°C which declines to 6.58 % at 27 °C and 1.72 % at 37 °C. Similar observations were made for other isolates like T_{III} reduced sulphide by 13.05 %, at 25°C. Isolate T_{IV} had percentage reduction of sulphide concentration by only 6.03 % at 37°C This is not expected and might be ascribed to the complex nature of tannery wastewater (Lofrano *et al.*, 2008; 2010) or genetic differences of the raw skins (Lefebvre *et al.*, 2005).

Increase McFarland standard from 0.5 – 1.0 McF, consistent reduction in sulphide (S²⁻) was observed through all the temperature regimes and period of treatment (Figures 4 – 6). The maximum percentage reduction of sulphide pollutants was 25.35 % and 20.42 % observed by day 5 with 1.0 McF at 25°C, for isolates T_{III} and T_V respectively, Figure 4 – 6. This is most probable because the carbon content of the wastewater that induced the reduction of sulphur may be depleting, which agrees with the literature reported by Lofrano *et al.* (2013; Zupančič and Jemec 2010)).

At day 9 with 1.0 McF, isolate T_{II} effected an increase in TDS by 51.09 % at 25°C and 64.03 % at 27°C, while isolate T_I had an increase in TDS to 81.67 % while, T_V to 80.12 % at 37°C as the highest reduction with the same condition.

RECOMMENDATION

The treatment process can further reduce TSS and sulphide concentration from the wastewater if a carbon source can be introducing into the treatment system to provide a source of energy for the anaerobic microorganisms. This is likely to prevent mutation between the microorganisms within the system. Further studies may therefore be necessary to establish the effect of introducing an energy source.

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CONCLUSION

Anaerobic treatment of sulphide-lime unhairing wastewater was observed to cause the reduction of sulphide by initiating the oxidation of carbon substances present in the wastewater thereby leading to the reduction of Sulphur to sulphide. Through this process anaerobic bacteria abstracting energy while, carbon substances get depleted. Some of these substances may be converted to methane gas, others to smaller substances thereby reducing the TSS leading to the reduction of the sludge expected from the sulphide-lime unhairing wastewater. The degradation action of anaerobes also causes the dissolution of the biodegradable wastewater and releasing traces of ions from their matrices causing an increase of the electrical conductivity of the treated wastewater.

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