EFFECT OF ACTI-ZYME LOADING, RETENTION TIME AND THEIR INTERACTION ON SEWAGE PHYSICOCHEMICAL PROPERTIES DURING ANAEROBIC TREATMENT

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ABSTRACT

Acti-zyme, an enzyme bio-catalyst was used for anaerobic sewage treatment. The sewage total Kjeldahl nitrogen (TKN), biochemical; oxygen demand (BOD_5), total suspended solids (TSS), total dissolved solids (TDS), electrical conductivity (EC), pH, chloride ions concentration (Cl^-), total phosphorous (TP), sulphate ions concentration (SO_4^{2-}), dissolved oxygen (DO) and the chemical oxygen demand (COD) were measured. All other parameters were measured by titrimetric methods; the pH and EC were measured by electrodes whereas the SO_4^{2-} ions were measured by the gravimetric method. Effects of the Acti-zyme loading, retention time and their interaction on sewage physicochemical properties were determined using 2^2 factorial designs. Samples were incubated at 37°C under anaerobic conditions at agitation of 60 rpm. Acti-zyme loading was varied between 35-50 g/m^3 whilst residence time was varied between 7-40 days. The sewage effluent TKN, BOD_5, TSS, EC, Cl^-, TP, SO_4^{2-} and COD decreased linearly >18% on increased Acti-zyme loading and residence time as well as their interactions unlike in Acti-zyme free effluent. The sewage pH changed from being alkaline to neutral and the DO increased by >200%. In overall, sewage treatment using Acti-zyme resulted in >90% decrease of the sewage contaminants. Highest sewage treatment conditions to meet the set guideline for disposal were obtained at 50 g/m^3 Acti-zyme loading and retention time of 40 days.

Keywords: Acti-zyme loadings, biological sewage treatment, physicochemical properties, retention times.

1.0 INTRODUCTION

Poorly treated sewage, a form of wastewater is being disposed off in water bodies in developing countries due to poor treatment techniques, lack of treatment chemicals or old equipment (Manyuchi and Phiri., 2013; Muserere *et al.*, 2014). This poses an environmental threat to the water bodies hence need to utilize environmentally friendly treatment techniques. Acti-zyme, an enzyme biocatalyst which possesses biochemical properties that allows it to be used for sewage treatment can be considered as an alternative sewage treatment method (Powell and Lundy., 2007; Tshuma, 2010). Acti-zyme contains enzymes such as catalase, protease and amylase which promote de-toxification of biological contaminants in sewage and has a potential to treat wastewater aerobically or anaerobically. Furthermore, anaerobic treatment of sewage is an attractive option as it can also favours biogas production and releases small quantities of sludge (Reali *et al*., 2001).

Acti-zyme was then applied for sewage treatment and the sewage effluent physicochemical parameters determined so as to ascertain the highest conditions in sewage treatment as a biological method.

2.0 MATERIALS AND METHODS

2.1 Materials

Raw sewage was collected from the Chitungwiza Sewage Treatment plant in the Mashonaland East area in Zimbabwe. Acti-zyme was obtained from AusTech Australia. An Inco Therm Labotec Incubator was used to hold the digesters. 250 mL flasks with a working volume of 190 mL were loaded with sewage. The flasks were first plugged with cotton wool then covered with aluminium foil paper to promote anaerobic conditions.

2.2 Methods

The raw sewage was stored at 0°C to avoid any microbial activity in the sewage. The raw sewage was tested for pH, total phosphorous (TP), total Kjeldahl nitrogen (TKN), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), electrical conductivity (EC), chloride ion concentration (CI^-), sulphates concentration (SO_4^{2-}) and the dissolved oxygen (DO) (Singh and Singh., 2010; Popa *et al*., 2012). The sewage was then treated with Acti-zyme loadings of 35-50 g/m^3 between 7-40 days (Powell and Lundy., 2007). All parameters were measured in milligrams per liter (mg/L) except for pH and the EC which was measured in microSiemens per centimeter (μS/cm). Temperature was fixed at 37◦C and agitation rate in the 250 mL flasks was maintained at 60 rpm. The TKN, TP, BOD_5, DO, COD, SO_4^{2-} and CI^- ions concentration were measured using titration methods, TSS and TDS were measured by filtration using the Alpha Standards Methods of determination in wastewater using a 20 µm filter (Alpha, 2005). The EC and pH were measured by the Hanna HI electrode probe. Lastly, the *E. Coli* content was measured through total plate count and the total coliforms by the spread count method. Sewage physicochemical properties were measured for Acti-zyme free (A_o) sewage effluent after 7 days and for effluent at varying Acti-zyme loadings and retention time (A_1) for thorough determination of the impact of Acti-zyme on sewage treatment.

STATISTICA 12.0 was used to check statistically if there was any interaction between Acti-zyme loading and the retention time on the various physicochemical properties at 95% confidence interval. 2^2 full factorial designs were done for each parameter to quantify the change in each parameter and the possible interactions (**Table 1)**. Acti-zyme loading was denoted as A and the residence time in the flasks was denoted as B. The (-1) and the (1) indicated the low and the high levels of the Acti-zyme loading and the residence time.

Table 1: 2^2 Factorial designs for determination of impact of Acti-zyme loading and retention time on sewage physicochemical properties

Experiment number	Treatment combinations		Conditions	
	A (Acti-zyme loading-g/m^3)	B (Retention time-days)	A	B
1	-1	-1	35	7
2	1	-1	50	7
3	-1	1	35	40
4	1	1	50	40

3.0 RESULTS AND DISCUSSION

All experiments were replicated thrice and the average values were used. Contour plots were generated in STATISTICA to represent the trends on the sewage effluent physicochemical properties with increasing Acti-zyme loadings and retention times. The plots were generated from day 7 of inoculating with Acti-zyme so that its effect can be quantified.

3.1 Raw sewage and treated effluent characteristics

The raw sewage physicochemical characteristics obtained are indicated in **Table 2**. All the physicochemical characteristics were off in terms of the body that govern effluents disposal in Zimbabwe i.e. the Environmental Management Agency (EMA) guidelines. This made the treatment of sewage using Acti-zyme essential. Although a decrease in the physicochemical properties was achieved for Acti-zyme free effluent (A_o) (**Table 2**), there was a clear indication that at 7 days and Acti-zyme loading of 35 g/m^3, the effluent with Acti-zyme's physicochemical parameters had decreased by more than 60% (**Table 2**) indicating the importance of sewage treatment with Acti-zyme to meet the set disposal guidelines.

3.2 Effect of Acti-zyme loadings, retention time and their interaction on sewage effluent physicochemical characteristics

Further work was then done in terms of quantifying the effect of Acti-zyme loading, retention time and their interaction for an acceptable effluent to be achieved. The trends on the sewage effluent physicochemical was then determined upon increasing Acti-zyme loading and retention time in the digesters. The regression models for determining each physicochemical parameter upon treatment with Acti-zyme were also given.

Table 2: Sewage characteristics for systems with Acti-zyme and without Acti-zyme in reference to disposal guidelines after 40 days

Parameter	Raw sewage	Sewage effluent (A_o)	Sewage effluent (A_1)	EMA Guidelines
TKN (mg/L)	245±5.5	39.9±0.20	9.4±0.36	10-20
BOD_5 @20°C (mg/L)	557±15.3	312.6±0.20	41.8±1.08	30-50
TSS (mg/L)	608±16.1	397.9±0.35	37.3±1.02	25-50
TDS (mg/L)	535±13	253.7±0.25	59.4±0.53	500-1500
E. Coli	TMC*	TMC*	TMTC	
EC @25°C (µS/cm)	3887±32.1	2186.2±0.21	1070.4±0.36	1000-2000
Cl^- (mg/L)	833±11.2	673.6±0.50	263.3±4.02	-
pH @25°C	9±0.3	7.9±0.20	6.3±0.1	6.0-9.0
Coliforms (cfu/ mL)	$1x10^{11}$	$1x10^{10}$	$1x10^{8}$	≤1000
TP (mg/L)	52±3.0	29.1±0.20	1.4±0.24	0.5-1.5
SO_4^{2-} (mg/L)	1192±70.8	776.9±0.35	53.6±2.71	-
DO (% Saturation)	7±0.2	20.9±0.26	87.0±0.20	≥ 60
Temperature (°C)	22±1.5	37±0.5	37±0.5	< 35
COD (mg/L)	738±12.6	409.5±0.38	77.9±2.24	60-90

* Too many to count

6.3.2.1 Effect of Acti-zyme loading and retention time on pH

pH measures the H^+ and OH^- ions in sewage. On treatment of sewage with Acti-zyme, the pH changed from being alkaline to almost neutral linearly with an R^2 value of 0.94. The change in pH varied from 9.0 to 6.3 in the sewage effluent with increased Acti-zyme loading and retention time in the sewage was 20% (**Figure 1**). Increase in Acti-zyme loading, increase in the retention time in the digester and their interaction had a positive effect on pH neutralization and this can be attributed to the removal of contaminants in the effluent due to Acti-zyme activity.

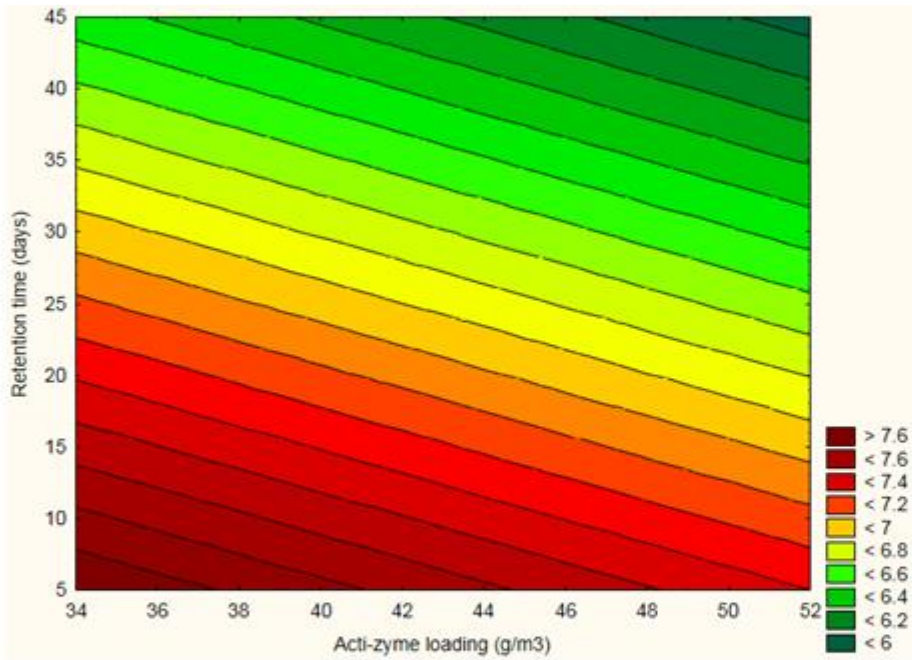

Figure 1: Effect of Acti-zyme loading and retention time on pH

6.3.2.2 Effect of Acti-zyme loading and retention time on TP

TP exists in sewage as phosphorous. The presence of phosphorus in raw sewage is found both as phosphates and as organically bound phosphorus and they have a potential to cause eutrophication if uncontrolled. The TP in the sewage effluent decreased linearly by an additional 19% with increase in Acti-zyme loading and the retention time with an R^2 value of 0.99 to around 1.4 mg/L (**Figure 2**). Increase in Acti-zyme loading, increase in the retention time and their interaction had a positive effect on the TP reduction possibly due to the uptake of nutrients in the sewage by Acti-zyme.

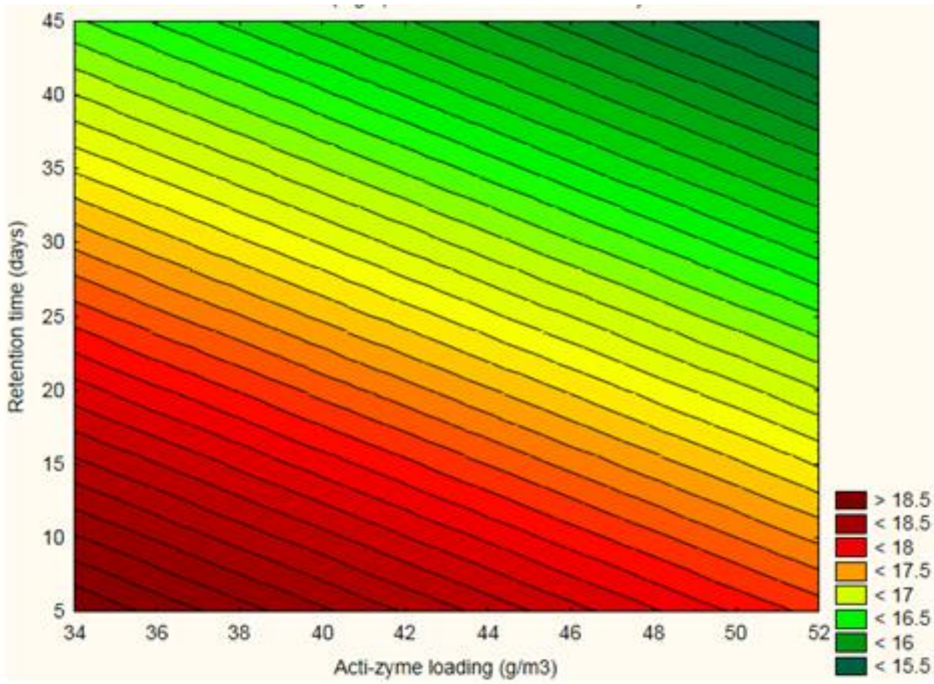

Figure 2: Effect of Acti-zyme loading and retention time on sewage TP

3.2.3 Effect of Acti-zyme loading and retention time on TKN

TKN is the measure of the Kjeldahl nitrogen which comprises of ammonia nitrogen and organically bound nitrogen. TKN if left uncontrolled have a potential to cause eutrophication and excessive nitrogen compounds release to the environment. Furthermore, the part of free ammonia of the total ammonia content is related increase in both water pH and temperature for the prevailing conditions in Zimbabwe with water temperatures > 20 ◦C. The amount of TKN in the sewage decreased linearly to 9.4 mg/L with increase in Acti-zyme loading and the retention time with an R^2 value of 0.96 (**Figure 3**). An additional 31% decrease was achieved from Acti-zyme loading of 35 g/m^3 to 50 g/m^3 at retention times of 7-40 days. Increase in Acti-zyme loading, increase in the retention time and their interaction had a positive effect on the TKN reduction.

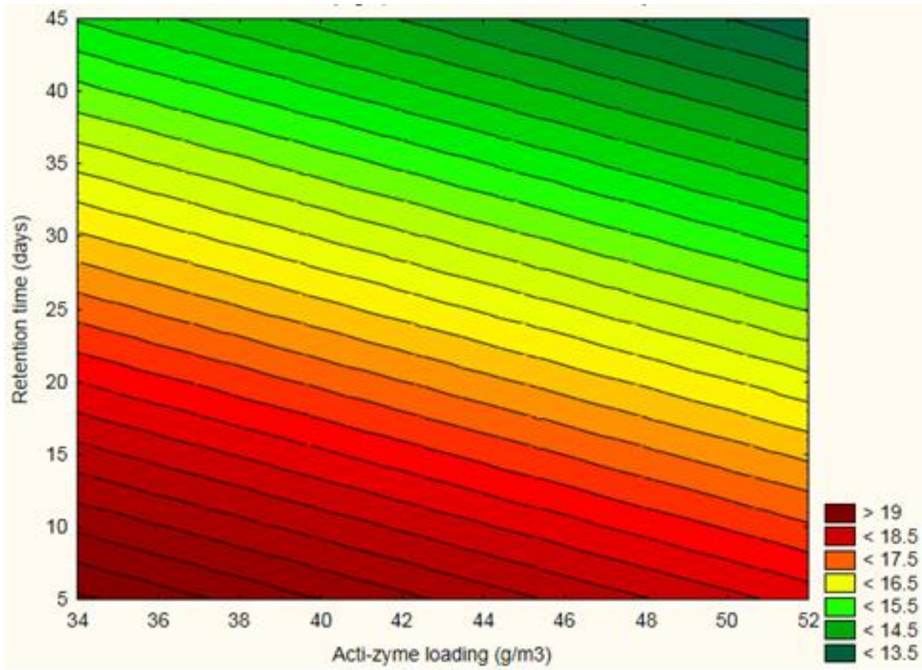

Figure 3: Effect of Acti-zyme loading and retention time on sewage TKN

3.2.4 Effect of Acti-zyme loading and retention time on BOD_5

BOD_5 refers to the amount of dissolved oxygen required by aerobic bacteria to break down organic matter. High BOD_5 and COD values negatively impact aquatic life in wastewater by lowering the oxygen levels in the wastewater killing aquatic life. High TKN and TP values can also increase the BOD_5. The BOD_5 in the sewage decreased linearly by an additional 33% to 41.8 mg/L with increase in Acti-zyme loading and the retention time with an R^2 value of 0.94 in the sewage effluent (**Figure 4**). The decrease in BOD_5 was attributed to the increased bio-degradation activity by Acti-zyme.

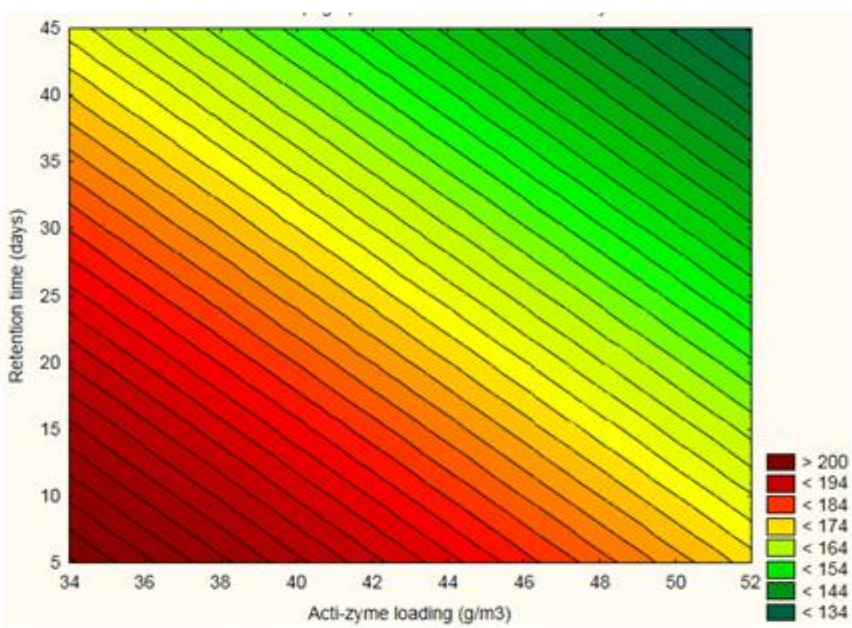

Figure 4: Effect of Acti-zyme loading and retention time on sewage BOD_5

3.2.5 Effect of Acti-zyme loading and retention time on COD

COD measures the amount of organic pollutants in sewage. The COD in the sewage decreased linearly by an additional 49% to 77.9 mg/L with increase in Acti-zyme loading and the retention time with an R^2 value of 0.94 in the sewage effluent (**Figure 5**). According to the ANOVA analyses with STATISTICA 12, only increase in Acti-zyme loading and increase in the retention time had a positive effect on the COD reduction but the interaction did not have a significant effect. The decrease is associated with Acti-zyme activity which promotes bio-degradation in sewage.

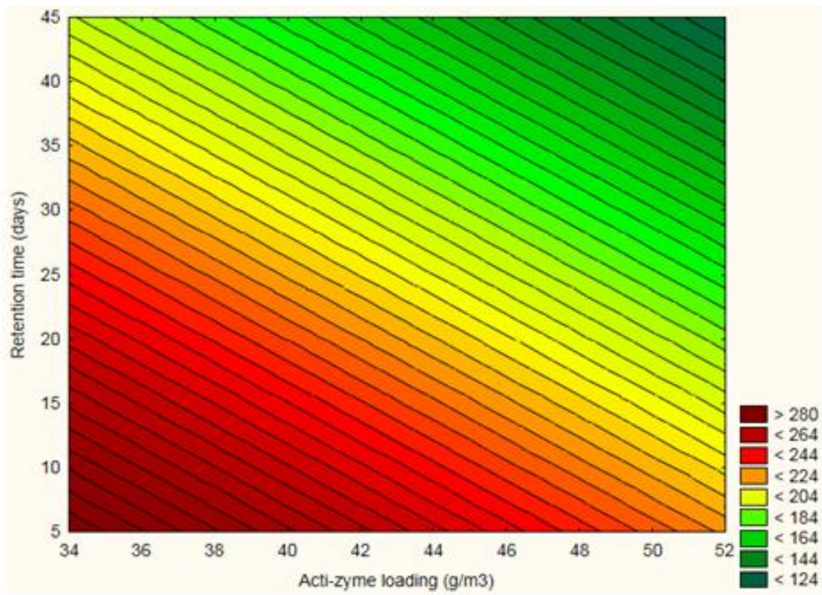

Figure 5: Effect of Acti-zyme loading and retention time on sewage COD

3.2.6 Effect of Acti-zyme loading and retention time on TSS

TSS measures the turbidity of water. High TSS and TDS values if uncontrolled can promote the limitation of dissolved oxygen available to aquatic life. The TSS in the sewage decreased linearly by an additional 55% to 37.3 mg/L with increase in Acti-zyme loading and the retention time with an R^2 value of 0.99 (**Figure 6**). According to the ANOVA analyses with STATISTICA 12, increase in Acti-zyme loading, increase in the retention time and their interaction had a positive effect on TSS reduction.

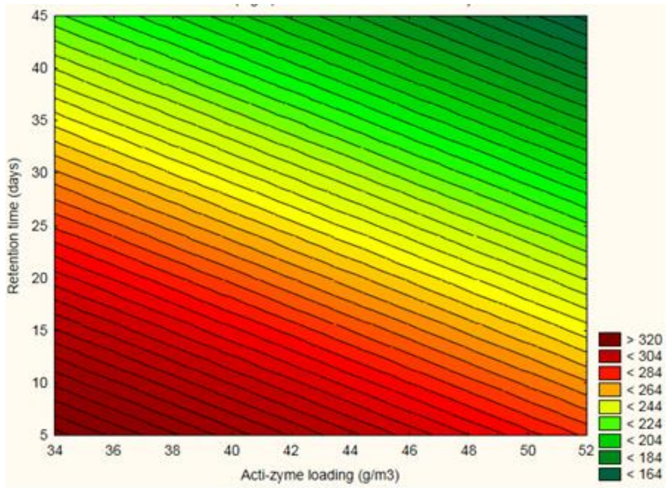

Figure 6: Effect of Acti-zyme loading and retention time on sewage TSS

3.2.7 Effect of Acti-zyme loading and retention time on TDS

TDS refer to the amount of mobile charged ions including minerals, salts and metals. The TDS in the sewage decreased linearly by an additional 26% to 59.4 mg/L with increase in Acti-zyme loading and the retention time with an R^2 value of 0.93 (**Figure 7**). The percentage decrease was 26 According to the ANOVA analyses with STATISTICA 12, only increase in Acti-zyme loading and increase in the retention time had a positive effect on TDS reduction. However, their interaction did not have an effect.

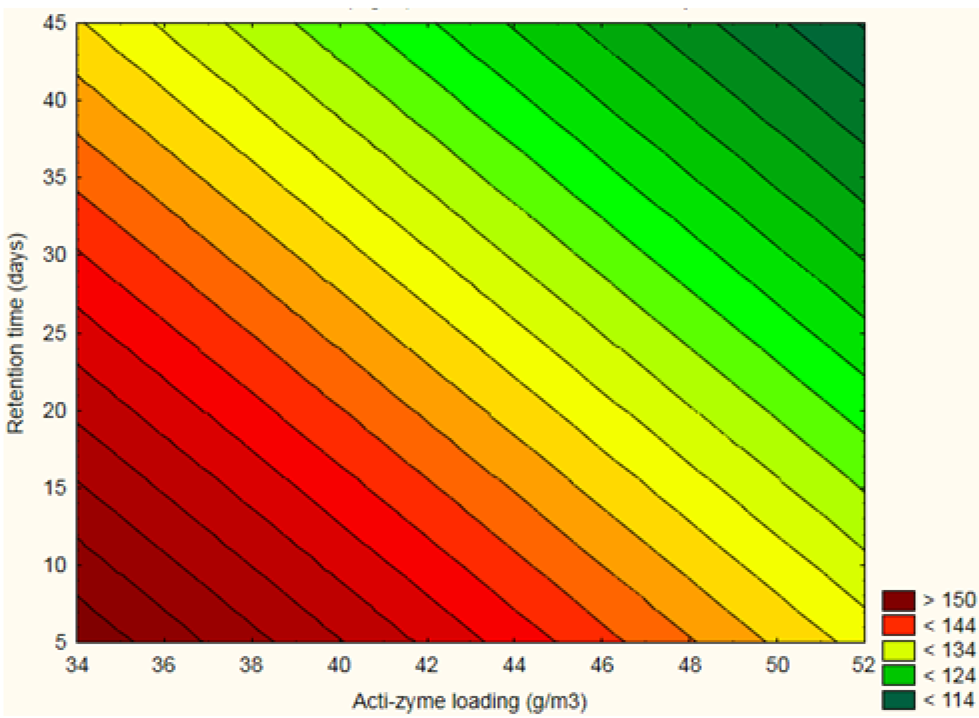

Figure 7: Effect of Acti-zyme loading and retention time on sewage TDS

3.2.8 Effect of Acti-zyme loading and retention time on EC

EC refers to the amount of dissolved ions in water that have the potential to conduct electricity. The EC is also directly affected by ions in wastewater such as chlorides, ammonia and sulphates. The EC in the sewage decreased linearly to by an additional 28% to 1070.4 μS/cm with increase in Acti-zyme loading and the retention time with an R^2 value of 0.97 (**Figure 8**). According to the ANOVA analyses with STATISTICA 12, increase in Acti-zyme loading, increase in the retention time in the digester and their interaction had a positive effect on the EC reduction. The change in TDS and the change in EC had a linear correlation with an R^2 value of 0.94 and had a correlation shown in **Equation 1**, whilst the pH and the EC had a linear correlation shown in **Equation 2** with an R^2 value of 0.98.

$$EC = 484.5pH - 922.62 \qquad (1)$$

$$TDS = 0.0467EC + 23.252 \qquad (2)$$

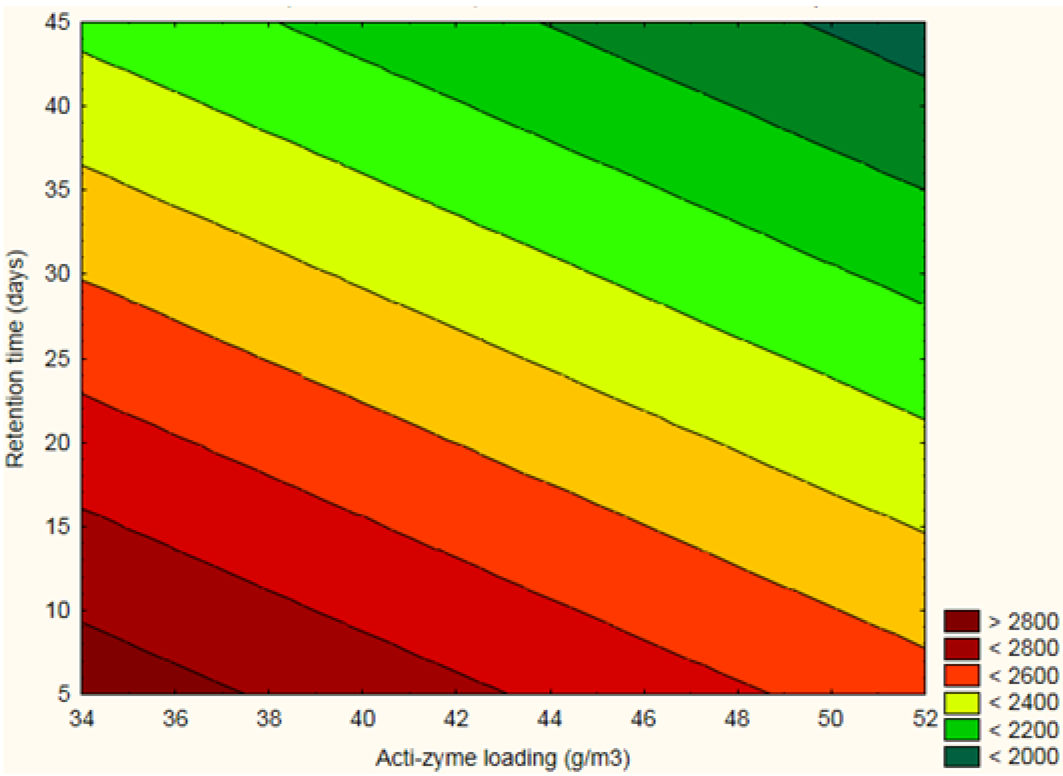

Figure 8: Effect of Acti-zyme loading and retention time on sewage EC

3.2.9 Effect of Acti-zyme loading and retention time on Cl^- ions concentration

The Cl^- ions concentration in the sewage decreased linearly to by an additional 56% to 263.3 mg/L in the sewage effluent with increase in Acti-zyme loading and the retention time with an R^2 value of 0.95 (**Figure 9**). According to the ANOVA analyses with STATISTICA 12, increase in Acti-zyme loading, increase in the retention time and their interaction had a positive effect on the Cl^- ions reduction.

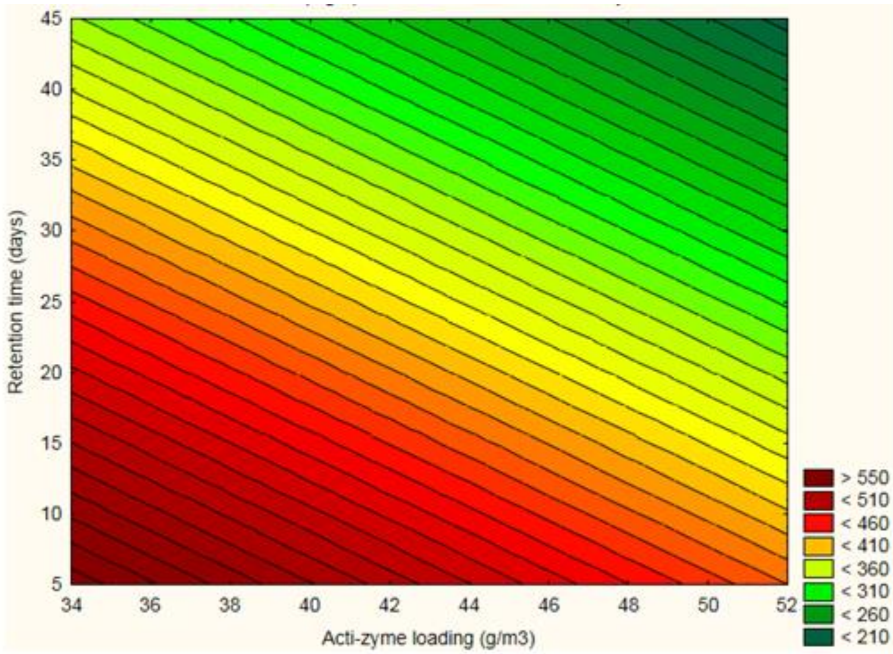

Figure 9: Effect of Acti-zyme loading and retention time on sewage Cl^- ions

3.2.10 Effect of Acti-zyme loading and retention time on SO_4^{2-} ions concentration

The SO_4^{2-} ions concentration in the sewage decreased linearly by an additional 91% to 53.6 mg/L in the sewage effluent with increase in Acti-zyme loading and the retention time with an R^2 value of 0.99 (**Figure 10**). This decrease can be attributed to the H_2S production hindering activities of Acti-zyme (Manyuchi *et al*., 2015). According to the ANOVA analyses with STATISTICA 12, only increase in Acti-zyme loading, increase in the retention time had a positive effect on the SO_4^{2-} ions reduction.

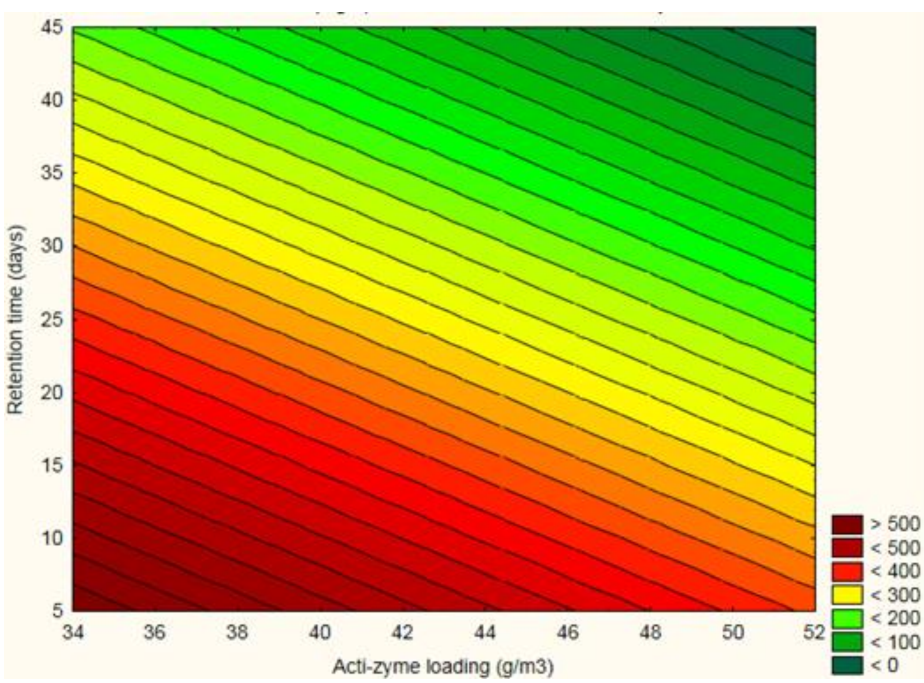

Figure 10: Effect of Acti-zyme loading and retention time on sewage SO_4^{2-} ions

3.2.11 Effect of Acti-zyme loading and retention time on DO

The DO concentration in the sewage effluent increased linearly to by an additional 222% to 87 mg/L with increase in Acti-zyme loading and the retention time with an R^2 value of 0.99 (**Figure 11**). According to the ANOVA analyses with STATISTICA 12, increase in Acti-zyme loading, increase in the retention time and their interaction had a positive effect on the DO increase. This was attributed to the removal of all contaminants due to the Acti-zyme action.

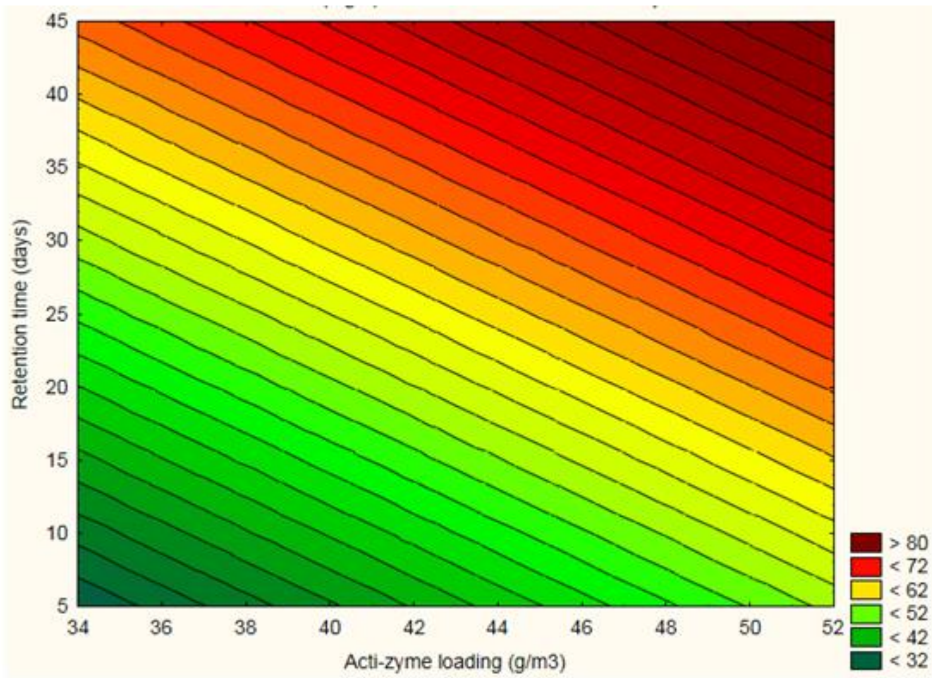

Figure 11: Effect of Acti-zyme loading and retention time on sewage DO

3.2.11 Effect of Acti-zyme Total E. Coli and coliforms content

The *E. Coli* in the sewage after treatment with Acti-zyme was too many to count. In addition, the total coliforms value was too high, that was around 10^8 coliforms. Despite, the *E. Coli* and total coliforms, the sewage effluent met the prescribed guidelines for sewage disposal either in accordance to EMA.

4.0 CONCLUSION

Acti-zyme effectively treats sewage removing all the wastewater contaminants to meet the required guidelines for effluent disposal in Zimbabwe compared to treatment without Acti-zyme. Sewage treated with Acti-zyme showed > 90% reduction in the sewage contaminants at highest loadings of 50 g/m^3 and retention time of 40 days. This makes Acti-zyme suitable for biological sewage treatment. However, chlorination must be done to remove the *E. Coli* and the total coliforms that will still be in treated effluent.

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