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Biodegradation Potential of Some Bacteria and Fungi Isolates from Septic Tank Sewage in Some Related Sites in Delta and Edo States of Nigeria

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Abstract

The biodegradation potentials of some selected bacteria isolated from septic tank sewage in the region under study were examined. Three replicate samples from Locations A, B and C representing Agbor, Benin and Sapele regions of Delta and Edo States, Nigeria respectively were collected from the influent tank (raw sewage) between November 2018 and January 2019 for testing. The sewage in 10 ml septic tubes for both experimental and control samples was subjected to shake flask biodegradation analysis using the isolates with the highest screen test result. A total of 18 bio-treatment options were available in this study. In the shake flask biodegradation of sewage using isolates, total organic carbon (TOC) values from day 0 to 25 in all isolates were lower compared with the control experiments. TOC values at day 25 were lowest (0.10 mg/L) in the treatment containing *Bacillus* sp. + *Klebsiella* sp. as compared to the TOC value (2.01 mg/L) of the treatment which had *Aeromonas* sp. + *Bacillus* sp. + *Klebsiella* sp. pH, EC, COD, TDS, DO and BOD were also monitored for a period of 3 weeks. In all treatment options, there was a rapid increase in the BOD for the experimental set up around day 10 - 20 which ranged between 74.82 - 187.54 mg/L. The overall physical observation (odour, consistency and colour) of the experimental and the control septic tubes showed very high remarkable reductions in the experimental set-up when compared with the observation made for the control samples.

Keywords

Biodegradation Potentials, Bacteria, Sewage, Septic Tank

1. Introduction

Septic tanks, activated sludge tanks, trickling filters and others sewage treatment plants (STPs) are examples of biological reactors that concentrate bacteria in conditions designed to encourage their proliferation and activity (Bounds, 1997; Bounds, 2001; Briones & Raskin, 2003). They reduce viable concentration of faecal bacteria such as coliforms and enterococci depending on the plant efficiency (Martins da Costa et al., 2006). However, relatively high number of bacteria still remain in STP effluents, up to 10^3 cfu/ml. Enterococci have also been reported (Caplin et al., 2008). Most of these organisms have been demonstrated to be able to utilize sewage (Conn et al., 2005; Crites & Tchnobanoglous, 1998).

Biological Oxygen Demand (BOD) which is a measure of the amount of oxygen required for the biochemical decomposition of biodegradable organic matter under aerobic conditions is perhaps the most common index in measuring sewage strength or concentration (Brake, 1998). Electrical conductivity (EC) and Total Dissolved Solids (TDS), are also useful parameters for assessing the concentration of solid substances present in any sample of waste water while pH, Dissolved Oxygen (DO) and Total Organic Carbon (TOC) like BOD is used in determining the strength of waste water.

EC is also known as the specific conductance. It is defined as a measure of the ability of a water sample to convey an electric current (Ademoroti, 1996; Tchnobanoglous & Kreiti, 2002). EC measures the total concentration of ionic solutes, while TDS measures the total amount of dissolved solids present in waste water (Adams, 1990). High values of EC show that inorganic ions such as H^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-} and HCO_3^- are present in reasonable concentration in the waste water. For TDS, high values obtained indicate that reasonable amount of solid matter is present in the waste water as dissolved substances. Rapid increases in BOD in waste water are usually attributed to high microbial population present in sewage, which requires greater oxygen consumption for the biodegradation of the sewage and hence the consequent decrease in dissolved oxygen content overtime. Thus, oxygen is a necessary requirement for aerobic degradation of waste water.

The shake flask has been used for decades to achieve aeration during an experimental set up design like the one used in this study for the cultivation of bacteria and fungi. They are an easy-to-use and inexpensive choice for basic applications such as organism screening, media design and early processes development. The agitation mode, direction and speed influence the fluid dynamics, and therefore the heat transfer and mass transfer in the culture, resulting in a specific performance (Buchs, 2001).

Microorganisms responsible for the decontamination of the septic tank range from bacteria to fungi to protozoa. Conventional municipal septic tank sewage systems are rarely reported in literature. However, one thing is notable, and that is, that septic tank systems are reputed for being of low efficiency of anaerobic biodegradation of organic matter, despite the diversity of microbes in the system. This paper studies the biodegradation potential of septic tank sewage inoculated with portions of single and consortia of microbial isolates previously obtained from a screening test, using the shake flask model as the experimental closed system.

2. Materials and Methods

2.1. Sample Collection

Sewage effluent samples were collected from different septic tanks within three locations in Edo/Delta States (Agbor, Benin and Sapele) in South-South region of Nigeria in November/December 2018 and January 2019 respectively; for physicochemical and microbiological analyses using 1 litre plastic bottles and 200 ml dark glass bottles. Samples were stored in ice packs (4˚C ± 2˚C) and transferred to the laboratory for analyses.

These septic tanks from the various locations (A, B and C) were chosen for their similarity in size and content. The only difference between the tanks was the number of persons served by the single tank and the life style of the users. Samples were collected in triplicates according to the standard methods for examination of water and waste water (APHA, 1995).

2.2. Biodegradation Potential of Microbial Isolates Using Shake Flask Degradation Tests

The organisms which showed the highest turbidity in the screening test was selected for shake flask degradation experiment according to the methods of Okpokwasili and Okorie (1988). Degradation of the waste water from septic tank was done using the isolates. It was assessed and monitored every 5 days for 25 days.

2.3. Screening Test for Septic Tank Effluent Utilizing Microorganisms

The method employed was adopted from Okpokwasili and Okorie (1988). The mineral salts medium was in volume 9.9 ml in separate 0.1 ml concentrated septic waste was added as amendment. All the test tubes were thereafter sterilized by autoclaving at 120˚C for 15 minutes after which they were allowed to cool. On cooling, each set of tube was inoculated with two drops of cell suspension of an isolate in sterile mineral salt broth. A set of control test tubes remained un-inoculated while the other control tubes had no septic waste. All test tubes were incubated at 28˚C ± 2˚C for 7 days, after which each tube was scored for turbidity (total viable count) that indicated utilization.

2.4. Shake Flask Biodegradation Test

One hundred and fifty millilitres (150 ml) of the mineral salt medium (MSM) was dispensed into different 250 ml conical flask in duplicates. Bacterial inoculant(s) were added to 10 ml of each concentrated septic waste sample in a test tube. These bacterial inoculants were earlier prepared by suspending a loop full each of the individual isolates in 2 ml mineral salt medium. Eighteen test tubes containing 10 ml septic waste samples were prepared. To four of these, single individual bacterial isolates of *Aeromonas* sp., *Bacillus* sp., *Enterococcus faecalis*, and *Klebsiella* sp. (A1, B, E, and K) respectively were prepared in separate 2 ml mineral salt media and added to each of the test tubes. A consortium of two isolates made up of two separate loop full single bacterium in 2 ml mineral salt medium; that is, (A1 + B), (A1 + E) (A1 + K), (B + E), (B + K) and (E + K), respectively were added to another six test tubes each containing 10 ml septic tank waste samples. Next, another four 10 ml test tubes samples had a consortium of three bacterial isolates with the following combinations: (A1 + E + K), (B + E + K), (A1 + B + E), (A1 + B + K) and respectively. Again, these were prepared by adding a loop full each of individual bacteria isolate to 2 ml mineral salt medium. Hence, 2 ml separate bacteria consortium will be added to each 10 ml test tube waste sample in the combinations stated above. Lastly, a consortium of bacteria designated as A1 + B + E + K was again prepared, each single bacterium isolate in 2 ml mineral salt medium. These were similarly transferred individually into another 10 ml septic tank waste sample in a test tube and mixed together. All test tube containing inoculants were subsequently introduced into the 250 ml conical flask containing 150 ml MSM. In all, there are a total of eighteen 250 ml conical flask samples with inoculants: 15 contained bacterial isolates, and 3 were fungal isolates. Of the three fungi samples, two contained separate fungus (*Aspergillus* sp. and *Penicillium* sp.) designated as (A2 and P) respectively; the third contained both fungi isolates together and designated as (A2 + P). Three conical flasks remained uninoculated, these were the control samples. All flasks were incubated at room temperature on a rotary shaker (Analox TM) operating at 12 rpm for 25 days. The total viable counts, pH, electrical conductivity, dissolved oxygen (DO) total dissolved solids (TDS) chemical oxygen demand (COD), biological oxygen demand (BOD) and total organic carbon (TOC) were monitored every five days and reported accordingly.

3. Results

Results obtained from the screening test of bacterial isolates from septic tank show that of the 9 isolates tested (Table 1) *Klebsiella* sp. and *Bacillus* sp. had the highest total viable counts after 7 days of incubation in mineral salt medium (MSM) (19.27×10^3 cfu/ml and 17.50×10^3 cfu/ml) respectively. *Staphylococcus* sp., *Micrococcus* sp. and *Proteus mirabilis* had the lowest total viable count (8.57×10^3 cfu/ml, 8.86×10^3 cfu/ml and 9.25×10^3 cfu/ml) respectively.

Other isolates including *Aeromonas* sp. (14.87×10^3 cfu/ml) and *Enterococcus faecalis* (11.22×10^3 cfu/ml) also had moderate total viable count during the screening test. Several fungal species were also found to have high total viable count during the screening test. These include *Aspergillus* sp. (9.60×10^3 cfu/ml) and *Penicillium* sp. (9.41×10^3 cfu/ml). A total of 6 isolates were used in the study;

Table 1. Screening test result of bacterial isolates from septic tank ($\times 10^3$ cfu/ml).

Isolates	Day 0	Day 7	Final result
Staphylococcus sp.	1.46	10.03	8.57
Aeromonas sp.	1.44	16.30	*14.87
Enterobacter aerogenes	1.46	13.79	*12.33
Bacillus sp.	1.42	18.92	*17.50
Enterococcus faecalis	1.50	12.72	*11.22
Escherichia coli	1.44	11.00	9.56
Klebsiella sp.	1.50	20.77	*19.27
Micrococcus sp.	1.54	10.40	8.86
Proteus mirabilis	1.48	10.73	9.25
Aspergillus sp.	1.38	10.98	*9.60
Penicillium sp.	1.36	10.77	*9.41

*Highest total viable counts.

4 bacteria (with high total viable count and moderate viable count in the screening test) and 2 fungi (with high total viable count in the screening test).

The biodegradation potential of the microbial isolates selected and used for the biodegradation of septic tank waste in mineral salt medium is shown in Tables 3-9. Results from the mean viable count (10^3 cfu/ml) show that within the 25 day period of treatment, the highest mean viable count was recorded around day 10 to day 15. This is in line with the growth curve theory for microbial species. *Bacillus* sp. had the highest mean viable count ($9.20 \pm 0.32 \times 10^3$ cfu/ml) while *Aeromonas* sp. had the least mean viable count of ($5.50 \pm 0.74 \times 10^3$ cfu/ml). *Aeromonas* sp., *Bacillus* sp., *Enterococcus faecalis*, *Klebsiella* sp., and an equal mixture of (*Aeromonas* sp. + *Bacillus* sp.), (*Aeromonas* sp. + *Enterococcus faecalis*), (*Aeromonas* sp. + *Klebsiella* sp.), (*Bacillus* sp. + *Enterococcus faecalis*), (*Bacillus* sp. + *Klebsiella* sp.), (*Enterococcus faecalis* + *Klebsiella* sp.), (*Aeromonas* sp. + *Enterococcus faecalis* + *Klebsiella* sp.), (*Bacillus* sp. + *Enterococcus faecalis* + *Klebsiella* sp.), (*Aeromonas* sp. + *Bacillus* sp. + *Enterococcus faecalis*), (*Aeromonas* sp. + *Bacillus* sp. + *Klebsiella* sp.), (*Aeromonas* sp. + *Bacillus* sp. + *Enterococcus faecalis* + *Klebsiella* sp.), *Aspergillus* sp., *Penicillium* sp. and (*Aspergillus* sp. + *Penicillium* sp.) were the 18 treatment options applied in the shake flask degradation test. pH, Electrical conductivity, COD, Total Dissolved Solids (TDS), DO, BOD and Total Organic Carbon (TOC) were also monitored for a period of 3 weeks.

4. Discussion

The results obtained from the screening test of bacterial isolates from septic tank show that of the 9 isolates tested, *Klebsiella* sp. and *Bacillus* sp. had the highest total viable counts after 7 days of incubation in mineral salts medium (MSM) (19.27×10^3 cfu/ml and 17.50×10^3 cfu/ml) respectively. *Staphylococcus* sp., *Mi-*

crococcus sp. and *Proteus mirabilis* had the lowest total viable count (8.57 × 10^3 cfu/ml, 8.86 × 10^3 cfu/ml and 9.25 × 10^3 cfu/ml) respectively.

Other isolates including *Aeromonas* sp. (14.87 × 10^3 cfu/ml) and *Enterococcus faecalis* (11.22 × 10^3 cfu/ml) also had minimal total viable count during the screening test. Several fungal species were also found to have high total viable count during the screening test. These included *Aspergillus* sp. and *Penicillium* sp. (Table 1).

Results from the shake flask degradation shows that the mean total viable count (TVC 10^3 cfu/ml) shows that within the 25 day period of treatment, the highest mean viable count was recorded around day 10 to day 15 (Table 2). This is in line with the standard bacterial growth curve theory. *Bacillus* sp. had the highest mean total viable count (9.20 ± 0.32 × 10^3 cfu/ml) while *Aeromonas* sp. had the least mean viable count of (5.50 ± 0.74 × 10^3 cfu/ml) (Table 2). *Aeromonas* sp., (A1) *Bacillus* sp., (B) *Enterococcus faecalis*, (E) *Klebsiella* sp., (K) and an equal mixture of *Aeromonas* sp. + *Bacillus* sp., (A1 + B) *Aeromonas* sp. + *Enterococcus faecalis*, (A1 + E) *Aeromonas* sp. + *Klebsiella* sp., (A1 + K) *Bacillus* sp. + *Enterococcus faecalis*, (B + E) *Bacillus* sp. + *Klebsiella* sp., (B + K) *Enterococcus faecalis* + *Klebsiella* sp., (E + K) *Aeromonas* sp. + *Enterococcus faecalis* + *Klebsiella* sp., (A1 + E + K) *Bacillus* sp. + *Enterococcus faecalis* + *Klebsiella* sp., (B + E + K) *Aeromonas* sp. + *Bacillus* sp. + *Enterococcus faecalis*, (A1 + B + E) *Aeromonas* sp. + *Bacillus* sp. + *Klebsiella* sp., (A1 + B + K) *Aeromonas* sp. + *Bacillus* sp. + *Enterococcus faecalis* + *Klebsiella* sp., (A1 + B + E + K) *Aspergillus* sp., (A2) *Penicillium* sp. (P) *and Aspergillus* sp. + *Penicillium* sp. (A2 + P) were the 18 treatment options applied in the shake flask degradation test (Tables 3-9).

Most of these organisms have been demonstrated to be able to utilize sewage. BOD which is perhaps the most common index in measuring sewage strength or concentration was reported within the 25 day period. In all treatment options, there was a rapid increase in the BOD for the experimental set up around day 10 - 20 which ranged between 74.82 - 187.54 mg/L. This was attributed to the high microbial population present in the sewage which requires greater oxygen consumption for the degradation of the sewage. However BOD values were low at

Table 2. Mean total viable count (10^3 cfu/ml) of selected microorganisms used for the biodegradation of septic waste in mineral salt medium.

Treatment (Day)	*Aeromonas* sp.	*Bacillus* sp.	*Enterococcus* faecalis	*Klebsiella* sp.	*Aspergillus* sp.	*Penicillium* sp.	Control
0	3.40 ± 0.09	3.30 ± 0.24	3.40 ± 0.12	3.50 ± 0.35	3.2 ± 0.09	3.0 ± 0.12	0.00
5	3.80 ± 0.009	4.10 ± 0.39	4.50 ± 0.43	3.80 ± 0.43	3.5 ± 0.32	3.5 ± 0.15	0.00
10	5.10 ± 0.74	8.00 ± 0.24	8.20 ± 0.50	5.20 ± 0.53	4.80 ± 0.75	4.00 ± 0.27	0.00
15	5.50 ± 0.74	9.20 ± 0.32	8.50 ± 0.55	5.60 ± 0.50	6.70 ± 0.63	6.00 ± 0.35	0.00
20	4.70 ± 0.52	6.00 ± 0.37	5.70 ± 0.32	5.50 ± 0.41	6.20 ± 0.48	5.3 ± 0.21	0.00
25	3.80 ± 0.57	4.90 ± 0.50	4.00 ± 0.41	3.90 ± 0.26	3.50 ± 0.52	2.90 ± 0.12	0.00

Means of replicates ± standard error.

Table 3. Biological Oxygen Demand (BOD_5 mg/L) of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	4.69 ± 0.84	8.58 ± 0.45	1.11 ± 0.12	7.99 ± 0.22	82.60 ± 0.32	5.25 ± 0.21	109.04 ± 0.75	3.45 ± 0.18	143.93 ± 0.40	2.26 ± 0.80	1.72 ± 0.38	1.70 ± 0.34
B	5.83 ± 0.23	8.58 ± 0.34	1.38 ± 0.20	7.99 ± 0.87	83.99 ± 0.76	5.25 ± 0.34	110.87 ± 0.32	3.45 ± 0.23	146.35 ± 0.45	2.26 ± 0.55	1.26 ± 0.39	1.70 ± 0.39
E	5.63 ± 0.65	8.58 ± 0.56	1.34 ± 0.25	7.99 ± 0.28	79.82 ± 0.23	5.25 ± 0.53	105.36 ± 0.61	3.45 ± 0.56	139.08 ± 0.39	2.26 ± 0.29	1.6 ± 0.19	1.70 ± 0.77
K	4.93 ± 0.49	8.58 ± 0.58	1.17 ± 0.17	7.99 ± 0.79	87.33 ± 0.33	5.25 ± 0.35	115.28 ± 0.15	3.45 ± 0.45	152.17 ± 0.78	2.26 ± 0.66	1.27 ± 0.80	1.70 ± 0.79
A1 + B	5.41 ± 0.31	8.58 ± 0.55	1.29 ± 0.19	7.99 ± 0.78	95.40 ± 0.15	5.25 ± 0.54	125.92 ± 0.91	3.45 ± 0.45	166.22 ± 0.29	2.26 ± 0.30	2.20 ± 0.19	1.70 ± 0.13
A1 + E	4.69 ± 0.84	8.58 ± 0.45	1.11 ± 0.12	7.99 ± 0.22	82.60 ± 0.32	5.25 ± 0.21	109.04 ± 0.75	3.45 ± 0.18	143.93 ± 0.40	2.26 ± 0.80	1.72 ± 0.38	1.70 ± 0.34
A1 + K	5.83 ± 0.23	8.58 ± 0.34	1.38 ± 0.20	7.99 ± 0.87	83.99 ± 0.76	5.25 ± 0.34	110.87 ± 0.32	3.45 ± 0.23	146.35 ± 0.45	2.26 ± 0.55	1.26 ± 0.39	1.70 ± 0.39
B + E	5.63 ± 0.65	8.58 ± 0.56	1.34 ± 0.25	7.99 ± 0.28	79.82 ± 0.23	5.25 ± 0.53	105.36 ± 0.61	3.45 ± 0.56	139.08 ± 0.39	2.26 ± 0.29	1.6 ± 0.19	1.70 ± 0.77
B + K	4.93 ± 0.49	8.58 ± 0.58	1.17 ± 0.17	7.99 ± 0.79	87.33 ± 0.33	5.25 ± 0.35	115.28 ± 0.15	3.45 ± 0.45	152.17 ± 0.78	2.26 ± 0.66	1.27 ± 0.80	1.70 ± 0.79
E + K	5.41 ± 0.31	8.58 ± 0.55	1.29 ± 0.19	7.99 ± 0.78	95.40 ± 0.15	5.25 ± 0.54	125.92 ± 0.91	3.45 ± 0.45	166.22 ± 0.29	2.26 ± 0.30	2.20 ± 0.19	1.70 ± 0.13
A1 + E + K	4.69 ± 0.84	8.58 ± 0.45	1.11 ± 0.12	7.99 ± 0.22	82.60 ± 0.32	5.25 ± 0.21	109.04 ± 0.75	3.45 ± 0.18	143.93 ± 0.40	2.26 ± 0.80	1.72 ± 0.38	1.70 ± 0.34
B + E + K	5.83 ± 0.23	8.58 ± 0.34	1.38 ± 0.20	7.99 ± 0.87	83.99 ± 0.76	5.25 ± 0.34	110.87 ± 0.32	3.45 ± 0.23	146.35 ± 0.45	2.26 ± 0.55	1.26 ± 0.39	1.70 ± 0.39
A1 + B + E	5.63 ± 0.65	8.58 ± 0.56	1.34 ± 0.25	7.99 ± 0.28	79.82 ± 0.23	5.25 ± 0.53	105.36 ± 0.61	3.45 ± 0.56	139.08 ± 0.39	2.26 ± 0.29	1.6 ± 0.19	1.70 ± 0.77
A1 + B + K	4.93 ± 0.49	8.58 ± 0.58	1.17 ± 0.17	7.99 ± 0.79	87.33 ± 0.33	5.25 ± 0.35	115.28 ± 0.15	3.45 ± 0.45	152.17 ± 0.78	2.26 ± 0.66	1.27 ± 0.80	1.70 ± 0.79
A1 + B + E + K	5.41 ± 0.31	8.58 ± 0.55	1.29 ± 0.19	7.99 ± 0.78	95.40 ± 0.15	5.25 ± 0.54	125.92 ± 0.91	3.45 ± 0.45	166.22 ± 0.29	2.26 ± 0.30	2.20 ± 0.19	1.70 ± 0.13
A2	4.69 ± 0.84	8.58 ± 0.45	1.11 ± 0.12	7.99 ± 0.22	82.60 ± 0.32	5.25 ± 0.21	109.04 ± 0.75	3.45 ± 0.18	143.93 ± 0.40	2.26 ± 0.80	1.72 ± 0.38	1.70 ± 0.34
P	5.83 ± 0.23	8.58 ± 0.34	1.38 ± 0.20	7.99 ± 0.87	83.99 ± 0.76	5.25 ± 0.34	110.87 ± 0.32	3.45 ± 0.23	146.35 ± 0.45	2.26 ± 0.55	1.26 ± 0.39	1.70 ± 0.39
A2 + P	5.63 ± 0.65	8.58 ± 0.56	1.34 ± 0.25	7.99 ± 0.28	79.82 ± 0.23	5.25 ± 0.53	105.36 ± 0.61	3.45 ± 0.56	139.08 ± 0.39	2.26 ± 0.29	1.6 ± 0.19	1.70 ± 0.77

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

Table 4. pH of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	6.87 ± 0.12	6.98 ± 0.54	5.76 ± 0.77	6.95 ± 0.44	4.95 ± 0.35	6.67 ± 0.65	4.26 ± 0.64	6.41 ± 0.14	3.66 ± 0.80	6.15 ± 0.22	3.58 ± 0.55	6.40 ± 0.44
B	6.64 ± 0.68	6.98 ± 0.89	5.63 ± 0.30	6.95 ± 0.67	4.84 ± 0.84	6.67 ± 0.88	4.16 ± 0.76	6.41 ± 0.12	3.58 ± 0.32	6.15 ± 0.50	3.52 ± 0.33	6.11 ± 0.65
E	6.79 ± 0.65	6.98 ± 0.75	5.58 ± 0.64	6.95 ± 0.80	4.88 ± 0.45	6.67 ± 0.29	4.20 ± 0.43	6.41 ± 0.39	3.61 ± 0.38	6.15 ± 0.16	3.56 ± 0.57	6.11 ± 0.16
K	6.66 ± 0.67	6.98 ± 0.98	5.72 ± 0.52	6.95 ± 0.34	4.92 ± 0.92	6.67 ± 0.65	4.23 ± 0.43	6.41 ± 0.42	3.64 ± 0.65	6.15 ± 0.22	3.60 ± 0.89	6.11 ± 0.43
A1 + B	6.59 ± 0.25	6.98 ± 0.18	5.59 ± 0.62	6.95 ± 0.59	4.81 ± 0.51	6.67 ± 0.31	4.13 ± 0.43	6.41 ± 0.12	3.56 ± 0.49	6.15 ± 0.30	3.45 ± 0.31	6.11 ± 0.23
A1 + E	6.74 ± 0.19	6.95 ± 0.19	5.47 ± 0.18	6.95 ± 0.60	4.70 ± 0.66	6.67 ± 0.39	4.05 ± 0.67	6.41 ± 0.64	3.48 ± 0.23	6.15 ± 0.70	3.48 ± 0.45	6.11 ± 0.46
A1 + K	6.87 ± 0.87	6.98 ± 0.17	5.54 ± 0.43	6.95 ± 0.22	4.76 ± 0.36	6.67 ± 0.43	4.10 ± 0.42	6.41 ± 0.67	3.52 ± 0.36	6.15 ± 0.56	3.48 ± 0.42	6.11 ± 0.43
B + E	6.64 ± 0.34	6.98 ± 0.23	5.49 ± 0.24	6.95 ± 0.23	4.72 ± 0.77	6.67 ± 0.24	4.06 ± 0.16	6.41 ± 0.24	3.49 ± 0.50	6.15 ± 0.34	3.45 ± 0.16	6.11 ± 0.45
B + K	6.89 ± 0.91	6.00 ± 0.50	5.55 ± 0.67	6.95 ± 0.43	4.77 ± 0.39	6.67 ± 0.33	4.10 ± 0.31	6.41 ± 0.50	3.53 ± 0.76	6.15 ± 0.16	3.50 ± 0.83	6.11 ± 0.24
E + K	6.46 ± 0.33	6.98 ± 0.56	5.46 ± 0.27	6.95 ± 0.30	4.70 ± 0.30	6.67 ± 0.19	4.04 ± 0.16	6.41 ± 0.29	3.47 ± 0.40	6.15 ± 0.12	3.41 ± 0.19	6.11 ± 0.39
A1 + E + K	6.88 ± 0.29	6.98 ± 0.39	5.47 ± 0.30	6.95 ± 0.33	4.70 ± 0.50	6.67 ± 0.49	4.05 ± 0.18	6.41 ± 0.20	3.48 ± 0.18	6.15 ± 0.15	3.44 ± 0.39	6.11 ± 0.29
B + E + K	6.54 ± 0.14	6.98 ± 0.14	5.35 ± 0.13	6.95 ± 0.97	4.60 ± 0.64	6.67 ± 0.66	3.96 ± 0.39	6.41 ± 0.41	3.40 ± 0.58	6.15 ± 0.33	3.36 ± 0.63	6.11 ± 0.12
A1 + B + E	6.83 ± 0.38	6.98 ± 0.89	5.29 ± 0.53	6.95 ± 0.96	4.55 ± 0.99	6.67 ± 0.11	3.91 ± 0.76	6.41 ± 0.41	3.36 ± 0.33	6.15 ± 0.51	3.34 ± 0.43	6.11 ± 0.11
A1 + B + K	6.76 ± 0.23	6.98 ± 0.27	5.41 ± 0.14	6.95 ± 0.23	4.65 ± 0.43	6.67 ± 0.14	4.00 ± 0.21	6.41 ± 0.27	3.44 ± 0.23	6.15 ± 0.21	3.42 ± 0.34	6.11 ± 0.27
A1 + B + E + K	6.69 ± 0.21	6.98 ± 0.29	5.38 ± 0.73	6.95 ± 0.21	4.63 ± 0.09	6.67 ± 0.42	3.98 ± 0.17	6.41 ± 0.42	3.42 ± 0.21	6.15 ± 0.48	3.40 ± 0.42	6.11 ± 0.21
A2	6.77 ± 0.37	6.98 ± 0.34	5.57 ± 0.21	6.95 ± 0.37	4.79 ± 0.53	6.67 ± 0.21	4.12 ± 0.37	6.41 ± 0.18	3.54 ± 0.21	6.15 ± 0.37	3.50 ± 0.44	6.11 ± 0.37
P	6.81 ± 0.42	6.98 ± 0.22	5.61 ± 0.38	6.95 ± 0.51	4.82 ± 0.42	6.67 ± 0.22	4.15 ± 0.32	6.41 ± 0.13	3.57 ± 0.42	6.15 ± 0.32	3.51 ± 0.22	6.11 ± 0.42
A2 + P	6.75 ± 6.98	6.98 ± 0.19	5.42 ± 0.25	6.95 ± 0.11	4.66 ± 0.25	6.67 ± 0.56	4.01 ± 0.39	6.41 ± 0.25	3.45 ± 0.34	6.15 ± 0.11	3.44 ± 0.56	6.11 ± 0.25

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

Table 5. Electrical Conductivity (EC µs/cm) of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	297.00 ± 0.22	220.00 ± 0.34	213.00 ± 0.11	219.00 ± 0.56	683.90 ± 0.54	505.99 ± 0.34	517.49 ± 0.53	383.33 ± 0.54	392.04 ± 0.19	290.40 ± 0.70	133.45 ± 0.45	326.00 ± 0.91
B	302.00 ± 0.98	220.00 ± 0.70	265.00 ± 0.77	219.00 ± 0.34	694.59 ± 0.90	505.99 ± 0.75	526.20 ± 0.87	383.33 ± 0.54	398.64 ± 0.32	290.40 ± 0.45	153.50 ± 0.88	326.00 ± 0.43
E	287.00 ± 0.78	220.00 ± 0.67	256.00 ± 0.34	219.00 ± 0.20	219.00 ± 0.20	505.99 ± 0.51	500.07 ± 0.89	383.33 ± 0.19	378.84 ± 0.84	290.40 ± 0.29	135.00 ± 0.35	326.00 ± 0.27
K	314.00 ± 0.31	220.00 ± 0.20	224.00 ± 0.24	219.00 ± 0.19	722.19 ± 0.22	505.99 ± 0.49	547.11 ± 0.47	383.33 ± 0.38	414.48 ± 0.14	290.40 ± 0.34	164.03 ± 0.16	326.00 ± 0.75
A1 + B	343.00 ± 0.76	220.00 ± 0.19	246.00 ± 0.12	219.00 ± 0.18	788.89 ± 0.81	505.99 ± 0.14	597.64 ± 0.80	383.33 ± 0.19	452.76 ± 0.19	290.40 ± 0.19	225.4 ± 0.39	326.00 ± 0.19
A1 + E	312.00 ± 0.29	220.00 ± 0.19	239.00 ± 0.28	219.00 ± 0.66	717.59 ± 0.18	505.99 ± 0.29	543.63 ± 0.39	383.33 ± 0.19	411.84 ± 0.29	290.40 ± 0.59	152.16 ± 0.28	326.00 ± 0.29
A1 + K	387.00 ± 0.23	220.00 ± 0.56	223.00 ± 0.75	219.00 ± 0.32	890.09 ± 0.71	505.99 ± 0.89	674.31 ± 0.71	383.33 ± 0.83	510.84 ± 0.71	290.40 ± 0.89	183.23 ± 0.67	326.00 ± 0.89
B + E	333.00 ± 0.45	220.00 ± 0.50	264.00 ± 0.19	219.00 ± 0.34	765.89 ± 0.34	505.99 ± 0.83	580.22 ± 0.67	383.33 ± 0.83	439.56 ± 0.76	290.40 ± 0.19	141.34 ± 0.76	326.00 ± 0.78
B + K	401.00 ± 0.31	220.00 ± 0.34	236.00 ± 0.16	219.00 ± 0.34	922.29 ± 0.12	505.99 ± 0.50	698.70 ± 0.39	383.33 ± 0.11	529.32 ± 0.78	290.40 ± 0.42	101.10 ± 0.45	326.00 ± 0.50
E + K	352.00 ± 0.76	220.00 ± 0.77	254.00 ± 0.16	219.00 ± 0.40	809.59 ± 0.90	505.99 ± 0.68	613.32 ± 0.37	383.33 ± 0.15	464.64 ± 0.55	290.40 ± 0.38	141.52 ± 0.28	326.00 ± 0.66
A1 + E + K	428.00 ± 0.39	220.00 ± 0.34	244.00 ± 0.23	219.00 ± 0.45	984.39 ± 0.39	505.99 ± 0.19	745.75 ± 0.75	383.33 ± 0.33	564.96 ± 0.69	290.40 ± 0.29	621.30 ± 0.32	326.00 ± 0.26
B + E + K	397.00 ± 0.65	220.00 ± 0.20	259.00 ± 0.56	219.00 ± 0.18	913.09 ± 0.30	505.99 ± 0.50	691.73 ± 0.68	383.33 ± 0.33	524.04 ± 0.24	290.40 ± 0.91	911.00 ± 0.17	326.00 ± 0.62
A1 + B + E	368.00 ± 0.86	220.00 ± 0.20	250.00 ± 0.50	219.00 ± 0.18	846.39 ± 0.46	505.99 ± 0.50	641.20 ± 0.41	383.33 ± 0.45	485.76 ± 0.57	290.40 ± 0.90	853.20 ± 0.54	326.00 ± 0.62
A1 + B + K	407.00 ± 0.62	220.00 ± 0.61	261.00 ± 0.38	219.00 ± 0.55	936.09 ± 0.47	505.99 ± 0.61	709.16 ± 0.55	383.33 ± 0.43	537.24 ± 00.61	290.40 ± 0.66	781.00 ± 0.23	326.00 ± 0.78
A1 + B + E + K	443.00 ± 0.66	220.00 ± 0.48	239.00 ± 0.32	219.00 ± 0.48	101.88 ± 0.97	505.99 ± 0.54	771.88 ± 0.66	383.33 ± 0.65	584.76 ± 0.54	290.40 ± 0.32	60.00 ± 0.65	326.00 ± 0.48
A2	281.00 ± 0.44	220.00 ± 0.53	239.00 ± 0.76	219.00 ± 0.44	646.29 ± 0.66	505.99 ± 0.82	489.61 ± 0.87	383.33 ± 0.76	370.92 ± 0.45	290.40 ± 0.66	533.00 ± 0.43	326.00 ± 0.53
P	269.00 ± 0.74	220.00 ± 0.73	243.00 ± 0.64	219.00 ± 0.59	618.69 ± 0.74	505.99 ± 0.53	468.71 ± 0.64	383.33 ± 0.74	355.08 ± 0.98	290.40 ± 0.74	244.71 ± 0.38	326.00 ± 0.64
A2 + P	305.00 ± 0.92	220.00 ± 0.62	265.00 ± 0.76	219.00 ± 0.83	701.49 ± 0.63	505.99 ± 0.51	531.43 ± 0.42	383.33 ± 0.56	402.60 ± 0.75	290.40 ± 0.42	225.62 ± 0.51	326.00 ± 0.83

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

Table 6. Chemical Oxygen Demand (COD mg/L) of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	119.28 ± 0.03	100.74 ± 0.44	98.01 ± 0.23	99.22 ± 0.87	92.37 ± 0.36	130.97 ± 0.65	70.77 ± 0.82	172.88 ± 0.50	65.42 ± 0.76	128.20 ± 0.30	47.45 ± 0.47	127.00 ± 0.56
B	121.90 ± 0.54	100.74 ± 0.60	99.66 ± 0.32	99.22 ± 0.55	92.55 ± 0.70	130.97 ± 0.54	73.65 ± 0.45	172.88 ± 0.44	69.21 ± 0.19	128.20 ± 0.65	52.27 ± 0.63	127.00 ± 0.74
E	117.76 ± 0.56	100.74 ± 0.98	94.71 ± 0.12	99.22 ± 0.40	95.02 ± 0.50	130.97 ± 0.32	75.02 ± 0.12	172.88 ± 0.18	67.83 ± 0.67	128.20 ± 0.28	53.50 ± 0.54	127.00 ± 0.12
K	129.92 ± 0.92	100.74 ± 0.74	103.62 ± 0.62	99.22 ± 0.22	93.78 ± 0.33	130.97 ± 0.30	80.55 ± 0.80	172.88 ± 0.88	68.32 ± 0.63	128.20 ± 0.87	58.00 ± 0.76	127.00 ± 0.39
A1 + B	113.16 ± 0.17	100.74 ± 0.18	102.90 ± 0.32	99.22 ± 0.17	93.83 ± 0.38	130.97 ± 0.65	79.29 ± 0.45	172.88 ± 0.17	66.67 ± 0.45	128.20 ± 0.18	42.22 ± 0.37	127.00 ± 0.15
A1 + E	133.84 ± 0.16	100.74 ± 0.40	102.96 ± 0.45	99.22 ± 0.55	93.90 ± 0.48	130.97 ± 0.34	79.40 ± 0.66	172.88 ± 0.48	66.20 ± 0.89	128.20 ± 0.13	45.90 ± 0.30	127.00 ± 0.30
A1 + K	129.34 ± 0.71	100.74 ± 0.65	116.10 ± 0.91	99.22 ± 0.18	93.25 ± 0.56	130.97 ± 0.21	72.29 ± 0.16	172.88 ± 0.65	67.03 ± 0.67	128.20 ± 0.42	47.00 ± 0.36	127.00 ± 0.71
B + E	121.44 ± 0.78	100.74 ± 0.67	109.89 ± 0.77	99.22 ± 0.34	94.05 ± 0.50	130.97 ± 0.76	71.47 ± 0.50	172.88 ± 0.76	52.74 ± 0.42	128.20 ± 0.23	53.70 ± 0.24	127.00 ± 0.50
B + K	125.08 ± 0.78	100.74 ± 0.83	120.30 ± 0.39	99.22 ± 0.56	95.08 ± 0.31	130.97 ± 0.42	79.61 ± 0.24	172.88 ± 0.56	66.69 ± 0.45	128.20 ± 0.12	46.44 ± 0.34	127.00 ± 0.31
E + K	124.46 ± 0.42	100.74 ± 0.55	116.16 ± 0.23	99.22 ± 0.18	93.33 ± 0.33	130.97 ± 0.77	72.40 ± 0.45	172.88 ± 0.29	62.16 ± 0.30	128.20 ± 0.29	54.20 ± 0.19	127.00 ± 0.30
A1 + E + K	131.76 ± 0.31	100.74 ± 0.75	128.40 ± 0.82	99.22 ± 0.22	89.49 ± 0.94	130.97 ± 0.79	63.72 ± 0.27	172.88 ± 0.86	55.32 ± 0.53	128.20 ± 0.28	62.13 ± 0.32	127.00 ± 0.27
B + E + K	132.09 ± 0.23	100.74 ± 0.47	131.01 ± 0.31	99.22 ± 0.45	72.93 ± 0.60	130.97 ± 0.79	60.27 ± 0.72	172.88 ± 0.27	51.32 ± 0.31	128.20 ± 0.39	54.30 ± 0.43	127.00 ± 0.72
A1 + B + E	115.00 ± 0.27	100.74 ± 0.19	121.44 ± 0.45	99.22 ± 0.23	86.30 ± 0.45	130.97 ± 0.67	61.6 ± 0.46	172.88 ± 0.29	59.31 ± 0.97	128.20 ± 0.28	47.37 ± 0.74	127.00 ± 0.72
A1 + B + K	120.06 ± 0.66	100.74 ± 0.56	105.82 ± 0.47	99.22 ± 0.62	89.68 ± 0.66	130.97 ± 0.43	64.38 ± 0.62	172.88 ± 0.25	63.38 ± 0.47	128.20 ± 0.78	45.00 ± 0.55	127.00 ± 0.56
A1 + B + E + K	117.30 ± 0.19	100.74 ± 0.13	92.73 ± 0.54	99.22 ± 0.54	86.19 ± 0.54	130.97 ± 0.73	62.97 ± 0.32	172.88 ± 0.73	54.72 ± 0.29	128.20 ± 0.13	47.75 ± 0.66	127.00 ± 0.54
A2	109.94 ± 082	100.74 ± 0.43	92.73 ± 0.23	99.22 ± 0.34	82.40 ± 0.64	130.97 ± 0.76	61.57 ± 0.44	172.88 ± 0.66	53.28 ± 0.53	128.20 ± 0.82	56.28 ± 0.34	127.00 ± 0.82
P	123.93 ± 0.64	100.74 ± 0.37	88.77 ± 0.43	99.22 ± 0.44	97.18 ± 0.13	130.97 ± 0.98	74.67 ± 0.73	172.88 ± 0.83	64.17 ± 0.38	128.20 ± 0.98	58.25 ± 0.28	127.00 ± 0.32
A2 + P	121.90 ± 0.33	100.74 ± 0.36	100.65 ± 0.42	99.22 ± 0.34	92.86 ± 0.24	130.97 ± 0.83	75.37 ± 0.24	172.88 ± 0.83	63.49 ± 0.42	128.20 ± 0.39	47.24 ± 0.42	127.00 ± 0.42

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

Table 7. Total Dissolved Solids (TDS mg/L) of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	334.71 ± 0.77	247.94 ± 0.23	253.57 ± 0.34	187.83 ± 0.44	192.10 ± 0.22	142.30 ± 0.44	145.53 ± 0.65	107.80 ± 0.33	108.63 ± 0.30	111.69 ± 0.16	101.00 ± 0.56	111.00 ± 0.23
B	340.35 ± 0.43	247.94 ± 0.50	257.84 ± 0.65	187.83 ± 0.54	195.33 ± 0.88	142.30 ± 0.88	147.98 ± 0.65	107.80 ± 0.67	135.15 ± 0.16	111.69 ± 0.30	132.45 ± 0.76	111.00 ± 0.60
E	323.44 ± 0.76	247.94 ± 0.43	245.03 ± 0.15	187.83 ± 0.30	185.63 ± 0.40	142.30 ± 0.71	140.63 ± 0.49	107.80 ± 0.81	130.56 ± 0.30	111.69 ± 0.68	113.15 ± 0.23	111.00 ± 0.11
K	353.87 ± 0.53	247.94 ± 0.94	268.09 ± 0.68	187.83 ± 0.87	203.10 ± 0.30	142.30 ± 0.42	153.86 ± 0.86	107.80 ± 0.17	114.24 ± 0.14	111.69 ± 0.60	107.33 ± 0.74	111.00 ± 0.40
A1 + B	386.56 ± 0.19	247.94 ± 0.18	292.85 ± 0.18	187.83 ± 0.18	221.85 ± 0.58	142.30 ± 0.19	168.07 ± 0.94	107.80 ± 0.77	125.46 ± 0.28	111.69 ± 0.21	122.27 ± 0.90	111.00 ± 0.70
A1 + E	351.62 ± 0.65	247.94 ± 0.49	266.38 ± 0.18	187.83 ± 0.76	201.80 ± 0.27	142.30 ± 0.20	152.88 ± 0.17	107.80 ± 0.39	121.89 ± 0.60	111.69 ± 0.15	118.20 ± 0.16	111.00 ± 0.49
A1 + K	436.14 ± 0.89	247.94 ± 0.43	330.41 ± 0.87	187.83 ± 0.76	250.31 ± 0.65	142.30 ± 0.42	189.63 ± 0.87	107.80 ± 0.43	113.73 ± 0.65	111.69 ± 0.71	111.00 ± 0.21	111.00 ± 0.65
B + E	375.29 ± 0.65	247.94 ± 0.42	284.31 ± 0.76	187.83 ± 0.67	215.38 ± 0.78	142.30 ± 0.45	163.17 ± 0.78	107.80 ± 0.23	134.64 ± 0.89	111.69 ± 0.83	115.75 ± 0.42	111.00 ± 0.76
B + K	451.92 ± 0.56	247.74 ± 0.91	342.36 ± 0.67	187.83 ± 0.91	259.37 ± 0.78	142.30 ± 0.34	196.49 ± 0.78	107.80 ± 0.12	120.36 ± 0.43	111.69 ± 0.78	111.90 ± 0.67	111.00 ± 0.12
E + K	396.70 ± 0.13	247.94 ± 0.12	300.53 ± 0.39	187.83 ± 0.83	227.67 ± 0.22	142.30 ± 0.39	172.48 ± 0.39	107.80 ± 0.29	129.54 ± 0.19	111.69 ± 0.13	116.00 ± 0.22	111.00 ± 0.32
A1 + E + K	482.35 ± 0.82	247.94 ± 0.24	365.42 ± 0.64	187.83 ± 0.87	276.83 ± 0.76	142.30 ± 0.24	209.72 ± 0.27	107.80 ± 0.47	124.44 ± 0.45	111.69 ± 0.67	122.13 ± 0.32	111.00 ± 0.19
B + E + K	447.41 ± 0.14	247.94 ± 0.47	338.95 ± 0.59	187.83 ± 0.79	256.78 ± 0.65	142.30 ± 0.47	194.53 ± 0.34	107.80 ± 0.99	132.09 ± 0.23	111.69 ± 0.11	114.71 ± 0.14	111.00 ± 0.12
A1 + B + E	414.73 ± 0.17	247.94 ± 0.49	314.19 ± 0.19	187.83 ± 0.66	238.02 ± 0.39	142.30 ± 0.43	180.32 ± 0.23	107.80 ± 0.87	127.50 ± 0.54	111.69 ± 0.12	125.30 ± 0.55	111.00 ± 0.65
A1 + B + K	458.68 ± 0.78	247.94 ± 0.62	347.49 ± 0.61	187.83 ± 0.78	268.25 ± 0.56	142.30 ± 0.78	199.43 ± 0.66	107.80 ± 0.47	133.11 ± 0.56	111.69 ± 0.62	114.00 ± 0.61	111.00 ± 0.55
A1 + B + E + K	499.25 ± 0.73	247.94 ± 0.73	239.91 ± 0.73	187.83 ± 0.32	283.53 ± 0.51	142.30 ± 0.48	217.07 ± 0.54	107.80 ± 0.17	130.05 ± 0.48	111.69 ± 0.65	74.12 ± 0.54	111.00 ± 0.29
A2	316.68 ± 0.76	247.94 ± 0.66	239.73 ± 0.32	187.83 ± 0.82	181.75 ± 0.87	142.30 ± 0.66	137.69 ± 0.53	107.80 ± 0.64	121.89 ± 0.87	111.69 ± 0.64	62.30 ± 0.53	111.00 ± 0.18
P	303.16 ± 0.53	247.94 ± 0.74	229.67 ± 0.83	187.83 ± 0.53	173.99 ± 0.25	142.30 ± 0.83	131.81 ± 0.38	107.80 ± 0.53	230.93 ± 0.22	111.69 ± 0.66	120.00 ± 0.46	111.00 ± 0.53
A2 + P	343.73 ± 0.46	247.94 ± 0.78	260.40 ± 0.56	187.83 ± 0.42	197.27 ± 0.54	142.30 ± 0.42	149.40 ± 0.93	107.80 ± 0.39	135.15 ± 0.93	111.69 ± 0.48	134.70 ± 0.42	111.00 ± 0.39

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

Table 8. Dissolved Oxygen (DO mg/L) of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	8.52 ± 0.05	8.76 ± 0.75	4.45 ± 0.34	8.14 ± 0.66	1.06 ± 0.43	7.83 ± 0.52	0	5.14 ± 0.23	0	3.38 ± 0.34	0	3.38 ± 0.80
B	10.60 ± 0.76	8.76 ± 0.66	5.54 ± 0.66	8.14 ± 0.23	1.32 ± 0.96	7.83 ± 0.34	0	5.14 ± 0.78	0	3.38 ± 0.31	0	3.38 ± 0.89
E	10.24 ± 0.76	8.76 ± 0.22	5.35 ± 0.88	8.14 ± 0.29	1.27 ± 0.87	7.83 ± 0.63	0	5.14 ± 0.29	0	3.38 ± 0.34	0	3.38 ± 0.38
K	8.96 ± 0.96	8.76 ± 0.76	4.68 ± 0.68	8.14 ± 0.54	1.11 ± 0.74	7.83 ± 0.84	0	5.14 ± 0.15	0	3.38 ± 0.39	0	3.38 ± 0.60
A1 + B	9.84 ± 0.62	8.76 ± 0.19	5.14 ± 0.59	8.14 ± 0.14	1.22 ± 0.12	7.83 ± 0.18	0	5.14 ± 0.15	0	3.38 ± 0.25	0	3.38 ± 0.49
A1 + E	9.56 ± 0.80	8.76 ± 0.29	5.00 ± 0..12	8.14 ± 0.45	1.19 ± 0.39	7.83 ± 0.18	0	5.14 ± 0.39	0	3.38 ± 0.36	0	3.38 ± 0.60
A1 + K	8.92 ± 0.14	8.76 ± 0.16	4.66 ± 0.20	8.14 ± 0.35	1.11 ± 0.24	7.83 ± 0.36	0	5.14 ± 0.24	0	3.38 ± 0.43	0	3.38 ± 0.67
B + E	10.56 ± 0.83	8.76 ± 0.16	5.52 ± 0.3	8.14 ± 0.31	1.31 ± 0.16	7.83 ± 0.34	0	5.14 ± 0.34	0	3.38 ± 0.45	0	3.38 ± 034
B + K	9.44 ± 0.42	8.76 ± 0.45	4.48 ± 0.11	8.14 ± 0.24	1.06 ± 0.16	7.83 ± 0.45	0	5.14 ± 0.42	0	3.38 ± 0.24	0	3.38 ± 0.16
E + K	10.16 ± 0.53	8.76 ± 0.32	5.31 ± 0.39	8.14 ± 0.14	1.26 ± 0.91	7.83 ± 0.78	0	5.14 ± 0.38	0	3.38 ± 0.39	0	3.38 ± 0.19
A1 + E + K	9.76 ± 0.67	8.76 ± 0.67	4.82 ± 0.28	8.14 ± 0.48	1.15 ± 0.50	7.83 ± 0.89	0	5.14 ± 0.28	0	3.38 ± 0.45	0	3.38 ± 033
B + E + K	10.36 ± 0.33	8.76 ± 0.85	1.25 ± 0.35	8.14 ± 0.42	1.19 ± 0.19	7.83 ± 0.38	0	5.14 ± 0.44	0	3.38 ± 0.84	0	3.38 ± 043
A1 + B + E	10.00 ± 0.12	8.76 ± 0.78	5.23 ± 0.34	8.14 ± 0.33	1.24 ± 0.33	7.83 ± 0.23	0	5.14 ± 0.45	0	3.38 ± 0.56	0	3.38 ± 0.43
A1 + B + K	10.44 ± 0.27	8.76 ± 0.34	5.45 ± 0.21	8.14 ± 0.14	1.30 ± 0.25	7.83 ± 0.38	0	5.14 ± 0.21	0	3.38 ± 0.34	0	3.38 ± 014
A1 + B + E + K	10.20 ± 0.09	8.76 ± 0.19	5.00 ± 0.42	8.14 ± 0.09	1.04 ± 0.19	7.83 ± 0.29	0	5.14 ± 0.32	0	3.38 ± 0.19	0	3.38 ± 019
A2	9.56 ± 0.21	8.76 ± 0.18	5.00 ± 0.23	8.14 ± 0.43	1.19 ± 0.44	7.83 ± 0.18	0	5.14 ± 0.34	0	3.38 ± 0.44	0	3.38 ± 0.43
P	9.72 ± 0.22	8.76 ± 0.42	5.08 ± 0.34	8.14 ± 0.13	1.21 ± 0.43	7.83 ± 0.32	0	5.14 ± 0.43	0	3.38 ± 025	0	3.38 ± 0.13
A2 + P	10.60 ± 34	8.76 ± 0.42	5.54 ± 0.39	8.14 ± 0.25	1.32 ± 0.11	7.83 ± 0.34	0	5.14 ± 0.11	0	3.38 ± 0.34	0	3.38 ± 0.56

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

Table 9. Total Organic Carbon (TOC mg/L) of the 18 treatment options.

*Treatment Options	Day 0	Control	Day 5	Control	Day 10	Control	Day 15	Control	Day 20	Control	Day 25	Control
A1	15.68 ± 0.17	17.28 ± 0.55	10.35 ± 0.65	16.81 ± 0.81	3.72 ± 0.71	16.36 ± 0.37	2.46 ± 0.71	15.91 ± 0.65	1.62 ± 0.20	15.49 ± 0.22	1.00 ± 0.13	10.50 ± 0.71
B	16.82 ± 0.67	17.28 ± 0.50	9.42 ± 0.43	16.81 ± 0.17	3.39 ± 0.65	16.36 ± 0.65	1.9 ± 0.56	15.91 ± 0.44	1.06 ± 0.20	15.49 ± 0.56	1.0 ± 0.20	10.50 ± 0.49
E	16.25 ± 0.34	17.28 ± 0.34	12.35 ± 0.65	16.81 ± 0.19	4.45 ± 0.16	16.36 ± 0.48	3.38 ± 0.49	15.91 ± 0.30	2.57 ± 0.57	15.49 ± 0.16	1.23 ± 0.24	10.50 ± 0.27
K	14.22 ± 0.23	17.28 ± 0.29	7.54 ± 0.56	16.81 ± 0.17	2.71 ± 0.73	16.36 ± 0.16	1.44 ± 0.44	15.91 ± 0.84	0.76 ± 0.78	15.49 ± 0.50	0.54 ± 0.56	10.50 ± 0.23
A1 + B	15.62 ± 0.18	17.28 ± 0.18	7.18 ± 0.80	16.81 ± 0.81	2.59 ± 0.19	16.36 ± 0.91	1.19 ± 0.65	15.91 ± 0.34	0.55 ± 0.29	15.49 ± 0.18	0.53 ± 0.33	10.50 ± 0.19
A1 + E	15.17 ± 0.67	17.28 ± 0.29	8.04 ± 0.19	16.81 ± 0.40	2.89 ± 0.12	16.36 ± 0.39	1.53 ± 0.18	15.91 ± 0.31	0.81 ± 0.77	15.49 ± 0.36	0.67 ± 0.50	10.50 ± 0.17
A1 + K	16.41 ± 0.34	17.28 ± 0.36	11.49 ± 0.42	16.81 ± 0.16	4.14 ± 0.21	16.36 ± 0.16	2.9 ± 0.24	15.91 ± 0.36	2.03 ± 0.42	15.49 ± 0.16	1.25 ± 0.56	10.50 ± 0.36
B + E	16.76 ± 0.42	17.28 ± 0.45	10.56 ± 0.50	16.81 ± 0.89	3.8 ± 0.24	16.36 ± 0.67	2.39 ± 0.24	15.91 ± 0.50	1.51 ± 0.16	15.49 ± 0.31	0.98 ± 0.89	10.50 ± 0.24
B + K	14.98 ± 0.78	17.28 ± 0.43	4.94 ± 0.42	16.81 ± 0.83	1.78 ± 0.11	16.36 ± 0.67	0.59 ± 0.11	15.91 ± 0.83	0.19 ± 0.41	15.49 ± 0.50	0.1 ± 0.43	10.50 ± 0.42
E + K	16.12 ± 0.65	17.28 ± 0.56	8.87 ± 0.19	16.81 ± 0.28	3.19 ± 0.55	16.36 ± 0.65	1.76 ± 0.37	15.91 ± 0.66	0.97 ± 0.56	15.49 ± 0.88	0.43 ± 0.31	10.50 ± 0.30
A1 + E + K	15.49 ± 0.54	17.28 ± 0.72	11.77 ± 0.71	16.81 ± 0.86	4.24 ± 0.44	16.36 ± 0.36	3.22 ± 0.23	15.91 ± 0.91	2.45 ± 0.43	15.49 ± 0.54	1.58 ± 0.59	10.50 ± 0.50
B + E + K	16.44 ± 0.45	17.28 ± 0.72	12.50 ± 0.67	16.81 ± 0.18	4.50 ± 0.45	16.36 ± 0.63	3.42 ± 0.23	15.91 ± 0.59	2.60 ± 0.22	15.49 ± 0.40	2.39 ± 0.23	10.50 ± 0.23
A1 + B + E	15.87 ± 0.56	17.28 ± 0.99	10.47 ± 0.39	16.81 ± 0.19	3.77 ± 0.30	16.36 ± 0.38	2.49 ± 0.39	15.91 ± 0.20	1.64 ± 0.23	15.49 ± 0.18	1.47 ± 0.49	10.50 ± 0.34
A1 + B + K	16.57 ± 0.55	17.28 ± 0.23	12.09 ± 0.25	16.81 ± 0.34	4.35 ± 0.23	16.36 ± 0.27	3.18 ± 0.14	15.91 ± 0.34	2.32 ± 0.14	15.49 ± 0.23	2.01 ± 0.21	10.50 ± 0.34
A1 + B + E + K	16. 19 ± 0.48	17.28 ± 0.32	11.38 ± 0.17	16.81 ± 0.48	3.26 ± 0.29	16.36 ± 0.13	1.83 ± 0.42	15.91 ± 0.48	1.02 ± 0.09	15.49 ± 0.21	0.75 ± 0.13	10.50 ± 0.21
A2	15.17 ± 0.64	17.28 ± 0.37	11.38 ± 0.45	16.81 ± 0.64	4.10 ± 0.21	16.36 ± 0.53	3.07 ± 0.21	15.91 ± 0.44	2.3 ± 0.23	15.49 ± 0.34	1.92 ± 0.21	10.50 ± 0.53
P	15.43 ± 0.37	17.28 ± 0.43	11.72 ± 0.44	16.81 ± 0.63	4.22 ± 0.22	16.36 ± 0.43	11.97 ± 0.42	15.91 ± 0.32	2.44 ± 0.62	15.49 ± 0.22	2.0 ± 0.42	10.50 ± 0.22
A2 + P	16.82 ± 0.52	17.28 ± 0.25	11.44 ± 0.11	16.81 ± 0.56	4.12 ± 0.33	16.36 ± 0.56	2.8 ± 0.25	15.91 ± 0.42	1.9 ± 0.53	15.49 ± 0.25	1.45 ± 0.42	10.50 ± 0.34

*A1 = *Aeromonas* sp., B = *Bacillus* sp., E = *Enterococcus faecalis*, K = *Klebsiella* sp., A2 = *Aspergillus* sp. and P = *Penicillium* sp.

the start of the experiment (Day 0 and 5) ranged from 1.12 - 5.83 mg/L and at the end of the set up (Day 25) with BOD value as low as 1 mg/L; the former being attributed to acclimatization of microbes to the system, and the latter being ascribed to the drastically depleted organic matter content (Table 3).

pH values in all treatments showed a significant drop along the treatment line (from day 0 - 25) when compared to the control (Table 4). It moved from being neutral to becoming acidic in all treatment options. Production of organic acids by acid-forming and hydrogen-forming bacteria tends to lower the pH of the septic tank due to acidogenesis (during which acid forming bacteria reduce complex organic matter to organic acids). Under normal conditions, this pH reduction is buffered by bicarbonate produced by methane-forming bacteria (Bitton, 1999). Electrical Conductivity (EC), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Dissolved Oxygen (DO) and Total Organic Carbon (TOC) followed similar decreasing trend as pH in all treatment options explored (Tables 5-9) (Uwidia & Ukulu, 2013).

It was observed that some of the treatments inoculated with a mixture of isolates had lower EC, COD, TDS, DO and TOC indicating an increase in biodegradation potential. The mixture of *Aeromonas* sp. + *Bacillus* sp. + *Enterococcus faecalis* + *Klebsiella* sp. had the lowest mean TDS at Day 25 (74.12 ± 076 mg/L) and TOC (0.75 ± 0.54 mg/L) suggesting a high reduction in organic solids present in the treatment. This trend was also observed in the work of Nwambo and Kehinde (2013) who reported from their study sewage degradation that the tanks inoculated with a mixture of selected strains seems to be much more efficient in the digestion of the sewage when compared to the tanks that received single seed cultures. Hoselti and Frost (1995) reported similar with their work on waste water stabilization ponds. However, a few others showed no significant increase in biodegradation potential and even in some cases recording a drop in its ability to degrade sewage. This may be due to competition and antagonism between isolates which is sometimes experienced in microbial communities.

The differences in the various parameters measured between the experimental and the control septic could be attributed to the activities of the cultured isolates inoculated into the experimental tubes. Again, from physical observation of the tubes, factors such as odour, consistency and colour showed very high remarkable reductions in the experimental when compared with the observations made for control.

5. Conclusion

Results obtained from the bio-treatment experiments of the septic tank waste water using the shake flask degradation test showed that some of the treatments inoculated with a mixture of isolates had lower electrical conductivity (EC), chemical oxygen demand (COD), total dissolved solid (TDS), dissolved oxygen (DO) and total organic carbon (TOC) when compared to a single bacterium, inferring an increase in biodegradation potential. Biodegradation of septic tank sewage by a mixed bacterial culture of *Aeromonas* sp., *Bacillus* sp., *Enterococcus faecalis* and *Klebsiella* sp. had the lowest mean TDS and TOC at day 25 suggesting a high reduction in organic solids present in the treatment.

Generally, a combination of more than one type of microorganism resulted in a dramatic decrease in both TDS and TOC values, which can be regarded as indexes for biodegradation in the 25 day period. In other words, as the number of microbial consortium increased so did the efficiency of the degradation process. Therefore, in the engineering management of septic tanks, there is need to consider the vital roles played by microorganisms. Consequently, in order to ensure complete mineralization of septic tank sewage, a consortium of organisms working in tandem will bring about the stabilization and clarification of organic matter present in waste water. Finally, all physicochemical parameters studied similarly decreased in values within the period under review.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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