Degradation of Nevirapine and Trimethoprim from Aqueous Solutions using Selected Microorganisms

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Abstract— *Together with pharmaceutical residues, personal care products encompassing prescription drugs, fragrances, and cosmetics have been detected in groundwater and other aquatic environments, hence compromising the quality of water. Their classification as micropollutants is due to their antibacterial resistance potential, persistence, and ecotoxicity. Biodegradation has been identified as a potential mechanism in their removal. The focus of this study focus was bioaugmentation; (Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeroginosa) to enhance the degradation of Nevirapine and Trimethoprim in model aqueous solutions. A liquid chromatography-tandem mass spectrometer (LC-MS/MS) was used to determine the pharmaceuticals. The efficacy of the bacterial strains to degrade selected drugs was evaluated by making the two drugs the sole source of energy and carbon. From the experimental data, the highest percentage biodegradation was recorded; Pseudomonas aeroginosa (86 %) and Staphylococcus aureus (79 %) for TMP and NVP respectively.*

Keywords— Biodegradation, efficacy, LC-MS/MS, model solutions, pharmaceutical.

I. INTRODUCTION

The occurrence of pharmaceutical residues in surface waters is an emerging environmental concern (Zhou, Lutovsky, Andaker, Gough, & Ferguson, 2013). The main sources of these residues include wastewaters from hospitals, drug production facilities as well as agriculture. Owing to growth in population, coupled with the emergence of new ailments, many pharmaceutical products are being manufactured today for the protection of humans and animals. Low concentrations of pharmaceutical residues have deleterious effects on aquatic biota. It also has adverse effects on human health (Wang, Hu, & Wang, 2018).

Some pharmaceutical residues are partially broken down by animals. However, most are eliminated in their original forms. The residual pharmaceutical compounds in animal manure can easily penetrate the terrestrial environment and are readily transported into aquatic environs through direct runoff and leaching. Recent studies have also revealed that many pharmaceuticals are degraded partially since most municipal wastewater treatment plants are not designed for the removal of pharmaceuticals (Chefetz, Mualem, & Ben-Ari, 2008).

Trimethoprim and Nevirapine are widely used antibiotics and anti-retroviral respectively. Different techniques have been used for their removal including biological, physical, and chemical processes. Amongst the physicochemical methods adopted include, sorption by special materials, advanced oxidation processes (AOP), and photodegradation (Basha *et al*., 2010; Chefetz *et al*., 2008; Klavarioti, Mantzavinos, & Kassinos, 2009). While biodegradation of trimethoprim and nevirapine has been reported in several publications and reviews, only a few microalgae, bacterial and fungal species have been found to degrade them (Göbel, McArdell, Joss, Siegrist, & Giger, 2007). Of great consideration in the removal of organic micropollutants from wastewater are their water solubility, tendency to volatilize, hydrophobicity, and biodegradability.

The dynamics of bacterial populations exposed to different concentrations of antibiotics have been examined and modeled in relation to the minimum inhibitory concentration (MIC). Without drugs, the growth rate of cells is higher than the death rate, and thus a bacterial population always grows. When drug concentration increases, as long as the concentration remains below the MIC, the growth rate is higher than the death rate and thus a population still grows, albeit at a slower rate. When the drug

concentration increases further and reaches the MIC, the growth rate becomes equal to the death rate, and the population size is maintained at a constant level. Only at drug concentrations above the MIC does a bacterial population decline (Magiorakos *et al*., 2011). In this study pure cultures of *Escherichia coli, Pseudomonas aeroginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* commonly found in the water were used to evaluate the biodegradability of Nevirapine and Trimethoprim.

II. MATERIALS AND METHODS

2.1 Microbial Assay Preparation

Microorganisms all American Type Culture Collection (ATCC); *Bacillus subtilis, Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeroginosa* (27853) were obtained from the botany laboratory at Jomo Kenyatta University of Agriculture and Technology. The microorganisms were cultured in an optimal nutrient medium to generate a healthy breed of microorganisms that can withstand the toxicity of the pharmaceuticals. Culturing was performed in a laminar flow hood to ensure a germ-free environment. The healthy isolates were then transferred into other plates and stored in a freezer. The medium used was Mueller–Hinton agar jells which was prepared in de-ionized water. Minimum mineral salt medium (MMSM) prepared in de-ionized water contained the following compounds; KH_2PO_4, NH_4Cl, $Na_2HPO_4.2H_2O$, $CaCl_2.2H_2O$, $FeCl_3.6H_2O$, $MgSO_4.7H_2O$, and K_2HPO_4. The medium's pH was thereafter adjusted to 7.0 and then autoclaved for 20 min at 121°C to kill the existing microorganisms (Sharma *et al*., 2020).

2.2 Acclimatization of Microorganisms to the Pharmaceuticals

Frozen microorganisms meant for future studies were removed and transferred into conical flasks containing minimum mineral salt medium together with some amount of glucose to alleviate the microorganisms from starvation. The pharmaceuticals were added to each flask in concentrations to be used for biodegradation experiments (0.5, 1.0, and 1.5 mg/ml). The microorganisms were allowed to grow in presence of the pharmaceuticals for several days and then removed and stored at a temperature of 4°C.

2.3 Evaluation of the Pharmaceuticals Tolerance on Microorganisms

Effects of pharmaceuticals concentration on the growth of the microorganisms were evaluated by inoculating acclimatized microorganisms into flasks containing a small amount of glucose and MMSM. This was preceded by the separate addition of Nevirapine and Trimethoprim drugs into these flasks in a range of 0.5-10 mg/ml upon which the flask's optical densities were measured at 600 nm.

2.4 Biodegradation Experiments

Into 500 ml conical flasks, (98.0) ml of MMSM spiked with different concentrations of the selected pharmaceuticals was added followed by corking to prevent contamination. Acclimatized bacterial isolate (2.0 ml) was then placed in each flask to make up a volume of 100 ml. To prevent possible photodegradation, the flasks were covered with aluminium foil. The temperature was set at 25°C with a rotating speed of 150 rpm.

2.5 Control Experiments

Three control experiments were set up: without the microorganisms to account for the drug's abiotic degradation, with dead biomass to account for sorption to the biomass and autoclaving at temperatures of 121°C to kill other microorganisms present (Al-Gheethi *et al*., 2019; Gauthier, Yargeau, & Cooper, 2010). For the autoclaving and non- autoclaving experiments, two model solutions were prepared in conical flasks containing 50% de-ionized water and 50% methanol and a concentration of 0.5 mg/L of each pharmaceutical. Flask A was autoclaved while flask B was not autoclaved. These flasks were taken under the same conditions as those involved in biodegradation experiments. 1.0 ml of each solution was filtered with a PVDF filter and put in an HPLC vial and its concentration was determined using LC-MS/MS.

2.6 Monitoring Bacterial Growth

Assessment of bacterial growth was done by diluting 1.0 ml of biodegradation contents in the flasks with 2.0 ml of deionized water and measuring the absorbance using a UV-vis spectrophotometer at a wavelength of 600 nm. This was done until the optical density began to decline.

2.7 LC-MS/MS Determination

Trimethoprim and Nevirapine were determined using a Waters Micromass Quattro Ultima mass spectrometer coupled with an 1100 Agilent series HPLC (USA). The experimental conditions were mobile phase 30 % methanol (B) and 70 %

deionized water (A) with a column Evo C18 (100 mm x 3.0 mm, 5 mm particle size 100 A^0) at a flow rate of 0.45 ml/min. The injection volume was 10 mL. External temperature was maintained at 40 °C.

III. RESULTS AND DISCUSSION

3.1 Evaluation of Tolerance to Pharmaceuticals by Microorganisms

The use of indigenous microorganisms and optimization of their biodegradation parameters such as concentration of microorganisms, temperature, pH, and time has a great influence on biodegradation efficiency. Furthermore, they enhance the quality of the degradation process without necessarily polluting the environment (Al-Gheethi *et al.*, 2019). To determine the levels of pharmaceuticals that could be tolerated by the microorganisms, each microorganism was subjected to various concentrations of the pharmaceuticals and their growth was monitored. Presence of glucose in the media results in the generation of enough biomass that adsorbs on target compounds. In effect, low concentrations of the pharmaceuticals were utilized coupled with a reduction in toxicity. However, under the conditions used (1 mg/ml of each drug) in the biodegradation experiments, the microorganisms were only minimally affected leading to a conclusion that lower concentrations should be used in the biodegradation experiments. The MIC used ranged from 0.5 mg/ml-1.5 mg/ml. Figures (1-8) illustrate the effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP/NVP on the growth of microorganisms.

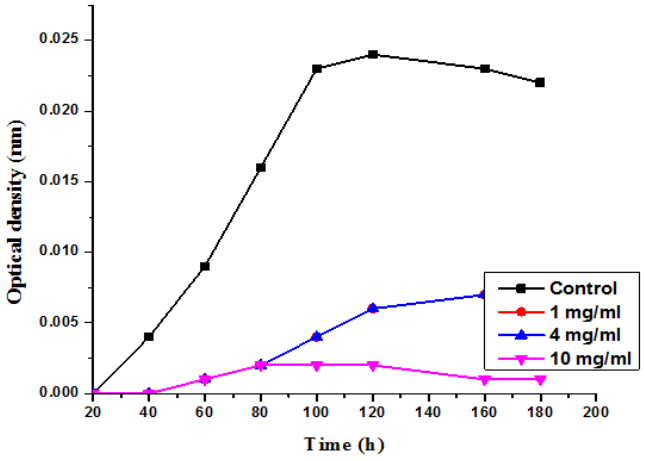

FIGURE 1: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *P. aeroginosa*

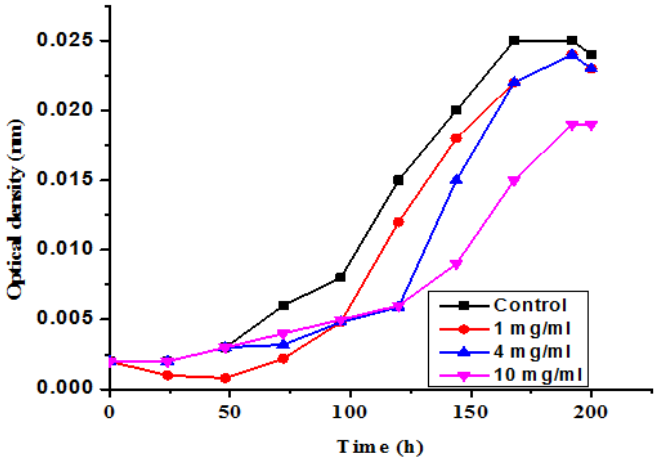

FIGURE 2: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *P. aeroginosa*

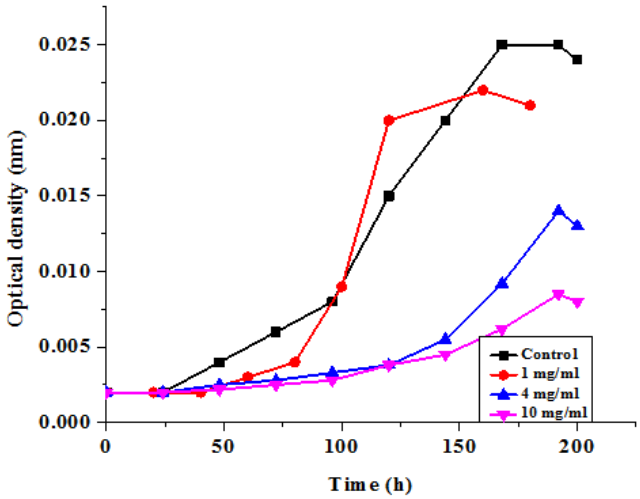

FIGURE 3: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *Bacillus subtilis*

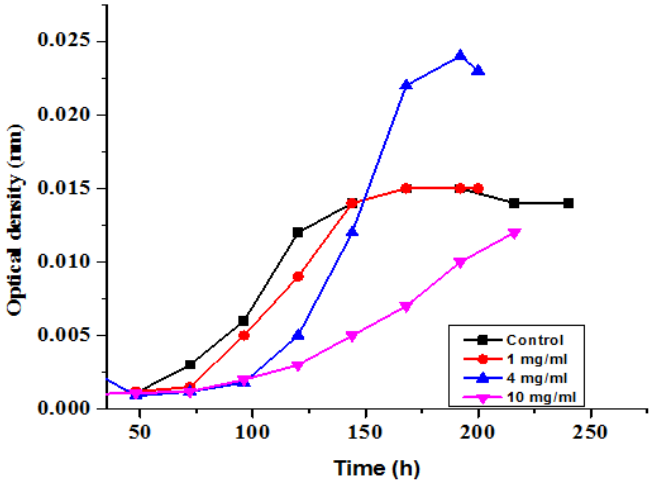

FIGURE 4: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *Bacillus subtillis*

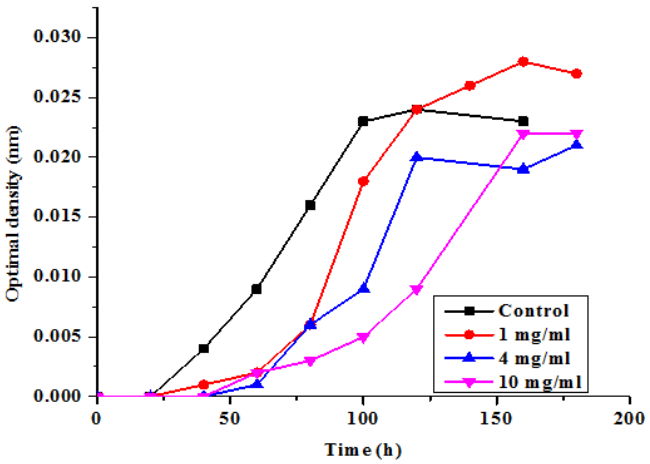

FIGURE 5: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *E. coli*

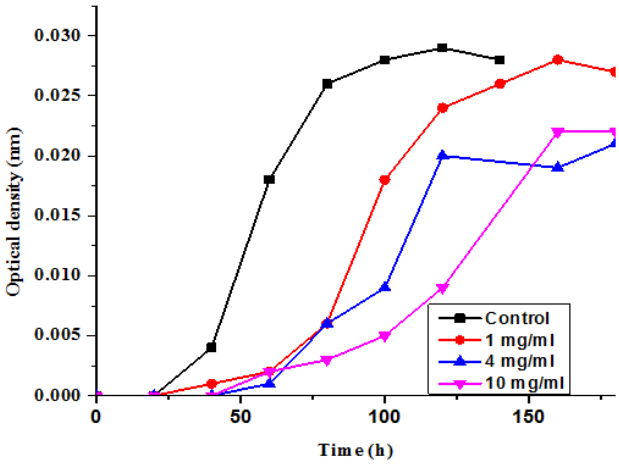

FIGURE 6: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *E.coli*

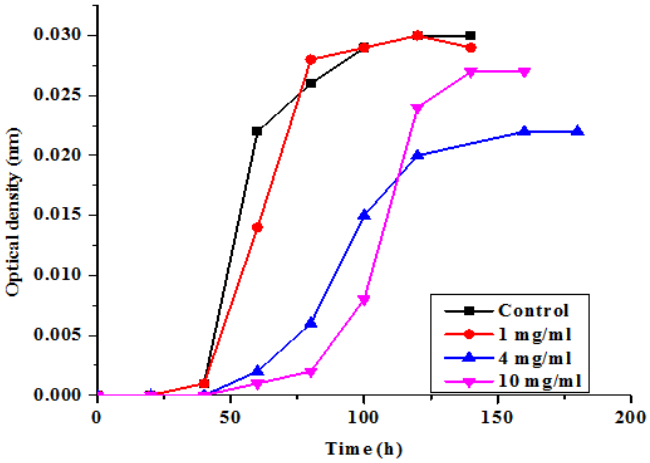

FIGURE 7: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *Staphylococcus aureus*

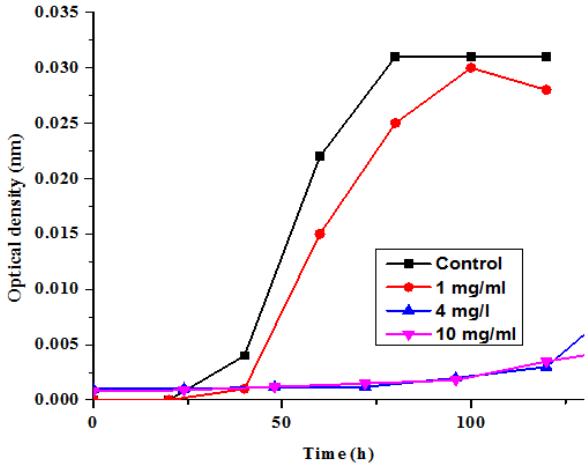

FIGURE 8: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *Staphylococcus aureus*

3.2 Biodegradation Studies

The biodegradation of pharmaceuticals was studied in 500 ml Erlenmeyer flasks for a period of one week and the percentage removal calculated (equation 1) after measuring the residual pharmaceuticals by LC-MS/MS. Representative chromatogram depicting the biodegradation of Trimethoprim and Nevirapine is shown on figure's 9 and 10 respectively.

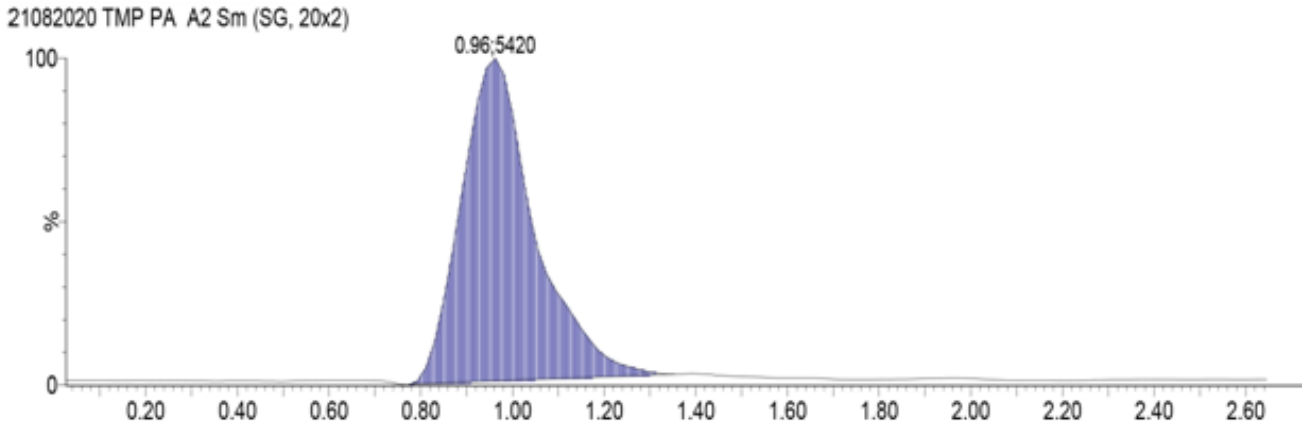

FIGURE 9: Biodegradation of Trimethoprim by *Pseudomonas aeroginosa*

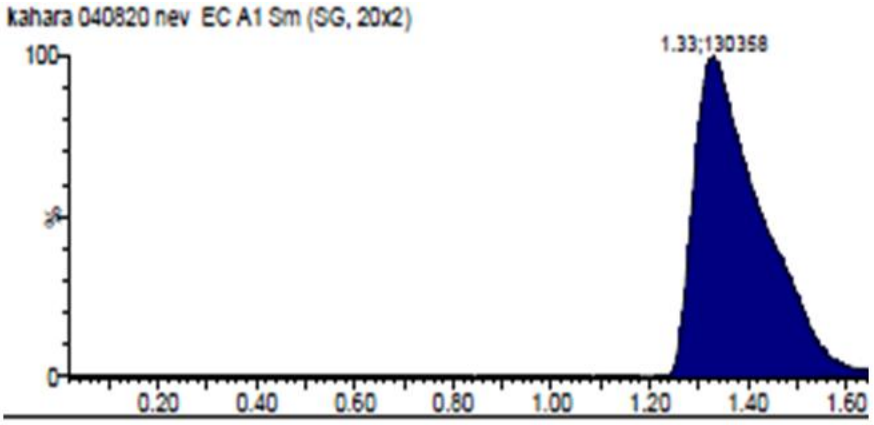

FIGURE 10: Biodegradation of Nevirapine by *E. coli*

Three important control experiments were set up: without the microorganisms to account for the drug's abiotic degradation, with dead biomass to account for sorption to the biomass, and autoclaving at temperatures of 121°C (Autoclaved TMP-0.44±0.02, Non-autoclaved TMP-0.42±0.01; Autoclaved NVP- 0.45±0.01, Non-autoclaved NVP- 0.43±0.01) to kill other microorganisms present *(Gauthier et al.,* 2010). From the control experiments, autoclaving had minimal effect on the biodegradation of the pharmaceuticals. Percentage biodegradation rate was much faster with *Staphylococcus aureus* (79 %) and least in *Pseudomonas aeroginosa* (35 %) for nevirapine over one week. Over the same time frame, with Trimethoprim, *Pseudomonas aeroginosa* (86 %) was the highest and least in *Bacillus subtilis* (73 %).

Equation 1 below was used to tabulate percentage biodegradation of the selected pharmaceuticals.

$$\text{Biodegradation } \% = \left(\frac{\text{Initia l Concentration} \quad - \text{Final Concentration}}{\text{Initial Concentration}}\right) \times 100 \tag{1}$$

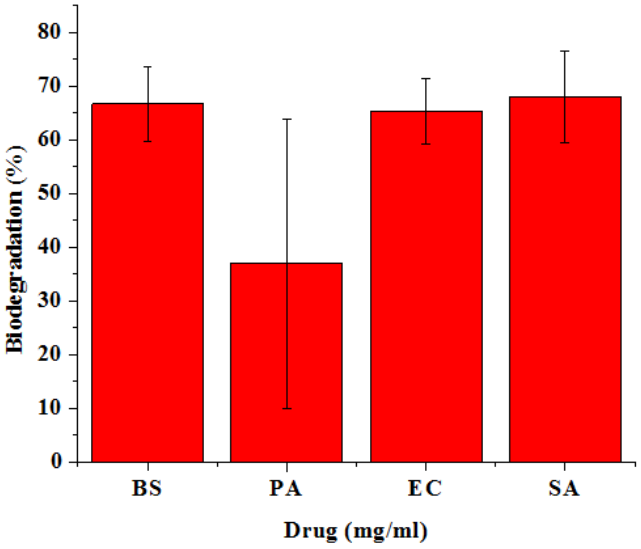

FIGURE 11: Percentage Biodegradation of Nevirapine with selected Microorganisms

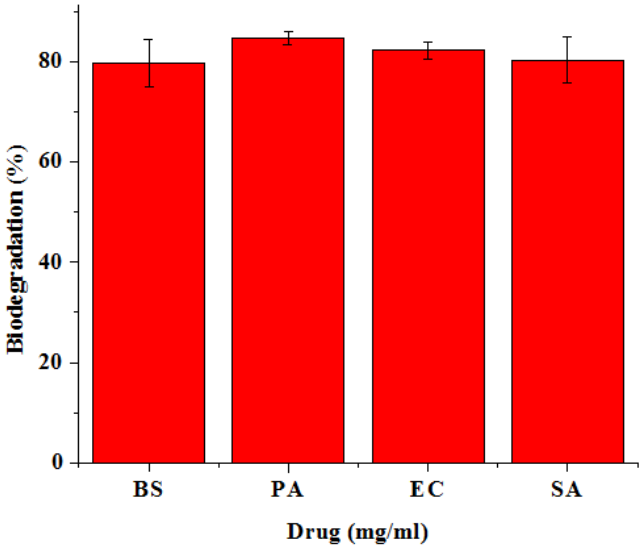

FIGURE 12: Percentage Biodegradation of Trimethoprim with selected Microorganisms

IV. CONCLUSION

Results show that *Pseudomonas aeroginosa, Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis* can degrade biodegrade. Highest percentage biodegradation were recorded for *Pseudomonas aeroginosa* 0.5 mg/ml (86 %) and *Staphylococcus aureus* 1.5 mg/ml (79 %), TMP and NVP respectively. Of late, the necessity for fresh biotechnological tools to get rid of pharmaceuticals from the environment, with less harm and negative impacts, has risen. Utilizing indigenous microorganisms can be a viable solution to tackle this menace. Based on the experimental data, it is evident that higher pharmaceutical concentrations have extreme effects on the microorganisms.

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