International Journal of Civil Engineering and Technology (IJCIET)
Volume 9, Issue 11, November 2018, pp. 2703–2713, Article ID: IJCIET_09_11_270
Available online at http://www.iaeme.com/ijciet/issues.asp?JType=IJCIET&VType=9&IType=11
ISSN Print: 0976-6308 and ISSN Online: 0976-6316

EFFECT OF CHICKEN FEATHER AND BACTERIAL TREATMENTS ON NITRITE LEVEL IN WASTEWATER

***Oghenerobor B. Akpor, Temitayo Arowolo and Olarewaju M. Oluba**
Department of Biological Sciences, Landmark University, PMB 1001, Omu-Aran, Kwara State, Nigeria
*Corresponding author

ABSTRACT

The aim of this study was to investigate the optimal conditions for nitrite in removal wastewater by two bacterial species (Pseudomonas aeruginosa and Bacillus subtilis) and chicken feather (raw and carbonated) fibre, under batch experimental conditions. Prior inoculating of the medium with the respective test bacterial species or addition of the feather fibres and every 24 h for 144 h duration, aliquot wastewater samples were withdrawn for the estimation of nitrite concentration using standard methods. The findings from this study revealed that nitrite removal from waste water is dependent on the pH, the initial concentration of nitrite in the media and the quantity of feather used or inoculum size of the test bacterial used for inoculation. In presence of the raw feather, optimum pH range for nitrite removal was observed to be between 6 and 12. No remarkable nitrite removal was observed in presence of the carbonated feather. Highest nitrite removal was observed with high inoculum concentration of the test bacterial species. At high initial nitrite levels in the medium, removal of nitrite was not observed in presence of the test bacterial species. The observation from this study could be employed in scale-up studies. In general, the study was able to provide valuable information on the role of chicken feather fibre and the test bacterial species in the removal of nitrite from wastewater under the experimental conditions investigated.

Keywords: Feather waste, nitrite removal, wastewater, microbes

Cite this Article: Oghenerobor B. Akpor, Temitayo Arowolo and Olarewaju M. Oluba, Effect of Chicken Feather and Bacterial Treatments on Nitrite Level in Wastewater, International Journal of Civil Engineering and Technology, 9(11), 2018, pp. 2703–2713

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1. INTRODUCTION

Nitrogen is an important element of nucleic acid which is present in all living cells. Nitrogen composites are available in food, manures, organic materials and also toxic substances. Nitrogen is vital to life, however its presence in excess amount in receiving water bodies can lead to eutrophication which is harmful to the environment [1].

Nitrite, a compound of nitrogen, is also used in food industry as a preservative, curing of meats and in meat products. It enhances the color and develops flavor in meats. It has been documented that sodium nitrite can prevent the growth of a wide variety of bacteria such as *Clostridia* and *Staphylococcus aureus*. The presence of excess nitrite in water bodies can lead to excess algal growth which will lead to eutrophication. The presence. Nitrite has also been known to be toxic to human health due to its oxidizing action on hemoglobin. It oxidizes hemoglobing in the blood to methemoglobin. The methemoglobin leads to a reduction of oxygen in the blood which causes a condition called methemoglobinemia [2].

The major effect of nitrite to humans is its involvement in the oxidation of normal hemoglobin to methemoglobin, which interferes with the ability of the body to transport oxygen to the tissues. The formation of methemoglobin is more susceptible in the infant than that of older children and adults. This is because the fetal hemoglobin present in the blood of infant is still high which can be easily oxidized to methemoglobin. Research has shown that a dose of nitrite causes a higher methemoglobin formation in infants than in adults [3].

To safeguard public health and maintain environmental sustainability, the concentration of nitrite in wastewater before discharge into receiving water bodies must be within the recommended limits. A variety of methods for nutrient removal from wastewater have been developed. Although chemical removal is said to be effective, due to its several drawbacks, biological processes are advocated in recent years. Apart from the use of microoragnsism, a variety of agrowastes have been indicated to be effective in nitrite reduction in water [4,5]. This study was therefore aimed at assessing the role of chicken feather fibre in nitrite removal from wastewater, in comaprison with removal in presence of two selecetd bacterial species.

2. MATERIAL AND METHODS

2.1. Chicken feathers and test bacterial species

Chicken feathers were collected from the commercial Farms of Landmark University, Omu-Aran, Kwara State, Nigeria. Before usage, the feathers were washed with detergent and rinsed thoroughly with water before being disinfected with 5% sodium hypochlorite. After disinfection, the feathers were sun-dried for one week and pulverized with a laboratory blender, then stored till when needed.

For the carbonation, known quantities of ground feather samples were kept in a crucible, placed in a laboratory furnace to carbonate at 250 ^{o}C for 2 h. After carbonation, samples were allowed to cool and stored until when needed.

Two bacterial species (*Pseudomonas aeruginosa* and *Bacillus subtilis*) were used for this study. Before use, the isolates were first streaked on nutrient agar plates, to ascertain their purity before sub culturing into broth cultures.

2.2. Experimental procedure

To a 250 mL capacity conical flask, 200 mL of wastewater (compounded 5g/L of sodium acetate and known quantity of sodium nitrite for setup involving the test bacterial species). The flasks containing the wastewater were sterilized in an autoclave at 121 ^{O}C for 15 min at 15 psi.

After sterilization, known quantities of the broth cultures of the bacterial species or chicken feather fibres were added to respective flasks and incubated at room temperature.

Immediately after inoculation and every 24 h, for a total duration of 144 h, aliquot wastewater samples were withdrawn from the respective flasks for estimation of nitrite concentration in the wastewater. The concentration of nitrite was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride to form a highly colored azo dye which was measured colorimetrically [6].

2.6. Statistical analysis

All experimental setups were carried out in duplicates. Statistical analysis was carried out using the SPSS Statistical Software. Comparison of means was determined using the One-Way Analysis of Variance (ANOVA) at 95 % confidence interval.

3. RESULTS

Nitrite level at 24 h of treatment in both control and carbonated feather treated wastewater was not significantly different ($p > 0.05$). However, at 48 h of treatment, the nitrite concentration in the carbonated feather treated water was significantly $p \leq 0.05$) higher compared to control. At 72 h, 96 h and 120 h, nitrite levels in the carbonated feather treated water were significantly decreased compared to the value recorded at 48 h but these values were comparatively higher than the observed concentration in the control water (Fig. 1).

In the raw feather set up, the observed nitrite levels in both control and the raw feather treated wastewater were not significantly different. However, following 72 h treatment period, significant increases in nitrite level were observed in the raw feather treated wastewater compared to the control. At 96 h, the nitrite concentration in the wastewater treated with 8 g raw feather showed a decrease, when compared to the control. At 120 h and 144 h, the 4 g, 6 g, and 8 g raw feather treated wastewater showed a dose-dependent significantly lower nitrite concentration compared to the control (Fig. 2).

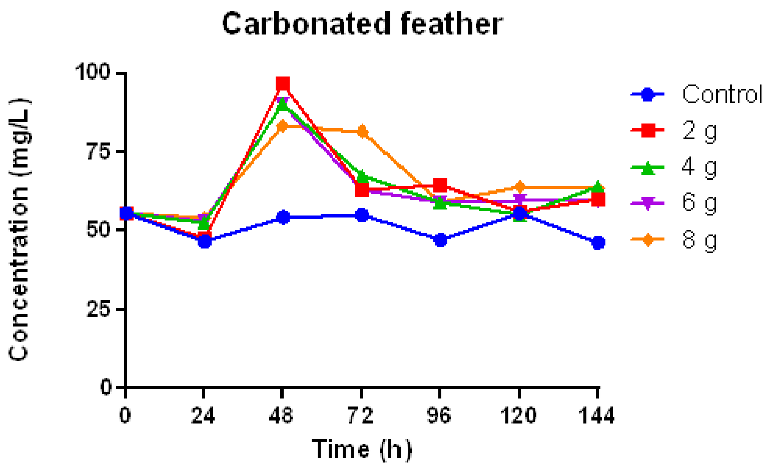

Figure 1: Changes in nitrite concentration in the wastewater in presence of varying quantities of the carbonated chicken feather

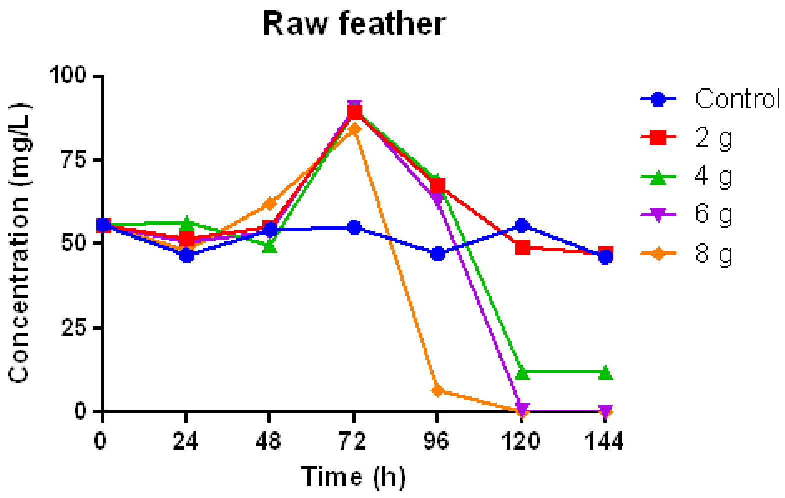

Figure 2: Changes in nitrite concentration in the wastewater in presence of varying quantities of the raw chicken feather

In the *Bacillus subtilis* set up, no significant change was observed in nitrite concentration in both control and *Bacillus subtilis* treated wastewater after 24 h. At 48 h and 72 h, the *Bacillus subtilis* treated wastewater showed significantly ($p < 0.05$) lower nitrite concentration compared to control. However, at 96 h and 120 h the nitrite levels in both control and the *Bacillus subtilis* treated groups were not significantly altered. A significant ($p < 0.05$) increase in nitrite concentration was observed in *Bacillus subtilis* treated groups compared to control at 144 h (Fig. 3).

For *Pseudomonas aeruginosa*, no significant alteration in nitrite concentration was noticed in both control and *Pseudomonas aeruginosa* after 24 h treatment. At 48 h, the 3 mL broth culture of *Pseudomonas aeruginosa* treated wastewater showed significantly ($p < 0.05$) higher nitrite level compared to control. However, at 72 h, the nitrite level in the *Pseudomonas aeruginosa* treated groups was observed not to be significantly different from the value observed for the control. At 96 h, the 2 mL broth culture of *Pseudomonas aeruginosa* treated wastewater had significantly ($p < 0.05$) higher nitrite concentration compared to control. At 120 h and 144 h, no observable significant change recorded in nitrite concentration in both *Pseudomonas aeruginosa* treated wastewater and the control (Fig. 4).

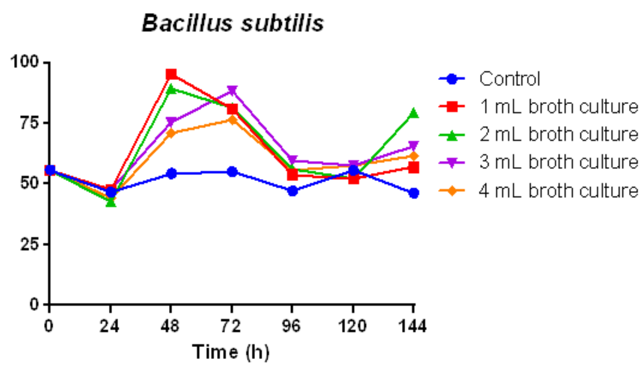

Figure 3: Changes in nitrite concentration in the wastewater in presence of varying quantities of the *Bacillus subtilis.*

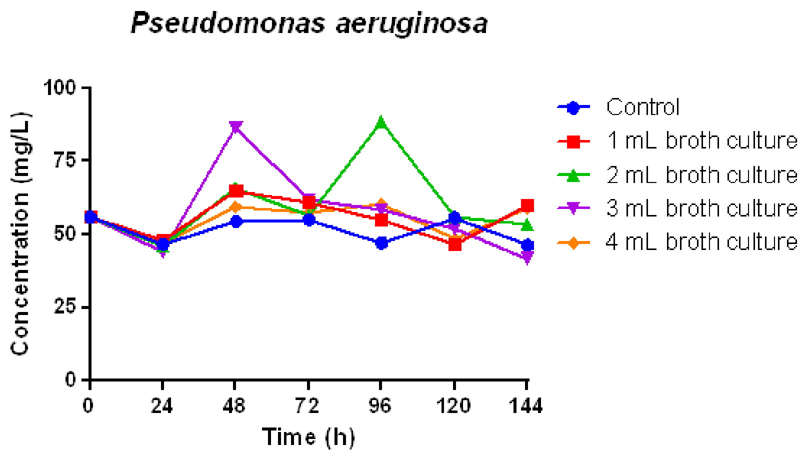

Figure 4: Changes in nitrite concentration in the wastewater in presence of varying quantities of the *Pseudomonas aeruginosa.*

In the carbonated feather treated wastewater, variation in pH produced no appreciable effect on the nitrite reducing potential throughout the treatment period. However, at 144 h, the nitrite level was observed to be significantly ($p < 0.05$) lower compared to the observed concentration at 0 h for pH 3 (Fig. 5).

In the experiment investigating the effect of pH on the nitrite reducing ability of raw chicken feather, a significant ($p < 0.05$) reduction in nitrite concentration in the wastewater was observed at pH 9 and 12 at 48 h and this trend was sustained till the end of the experiment at 144 h. On the other hand, pH 6 showed significant ($p < 0.05$) nitrite reducing potential after 96 h. No significant change in nitrite concentration was observed for raw feather treatment maintained at pH 3 (Fig. 6).

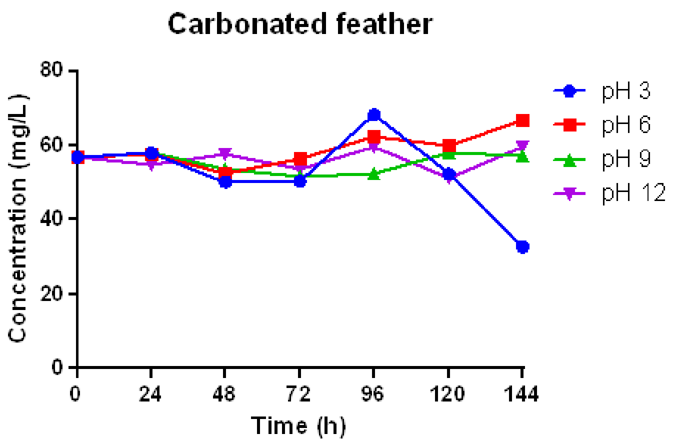

Figure 5: Changes in nitrite concentration in the wastewater in presence of the carbonated chicken feather at varying pH

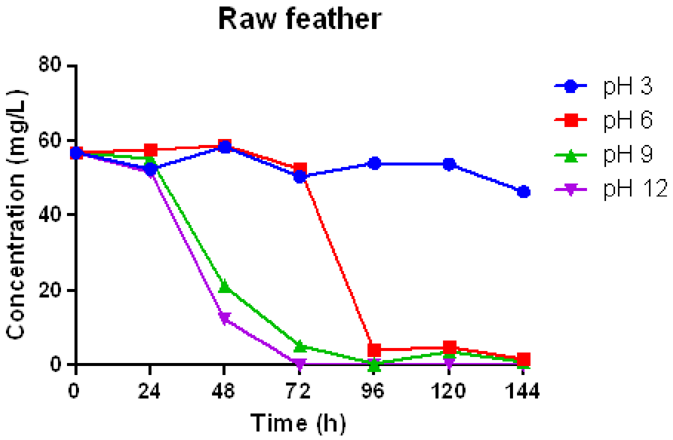

Figure 6: Changes in nitrite concentration in the wastewater in presence of the raw chicken feather at varying pH

The effect of pH on the nitrite reducing potential of *Pseudomonas aeruginosa* is represented in Fig.7 According to the results, *Pseudomonas aeruginosa* was observed to show potential for reducing nitrite concentration only at pH 6. Increasing the pH of the system was observed to be of no effect in the nitrite reducing ability of *P. aeruginosa* (Fig. 7).

The effect of pH on the nitrite reducing potential of *Bacillus subtilis* is represented in Fig. 4 According to the result, *Bacillus subtilis* was observed to show potential for reducing nitrite concentration only at pH 6. Increasing the pH of the system was observed to be of no effect in the nitrite reducing ability of *Bacillus subtilis* (Fig. 8).

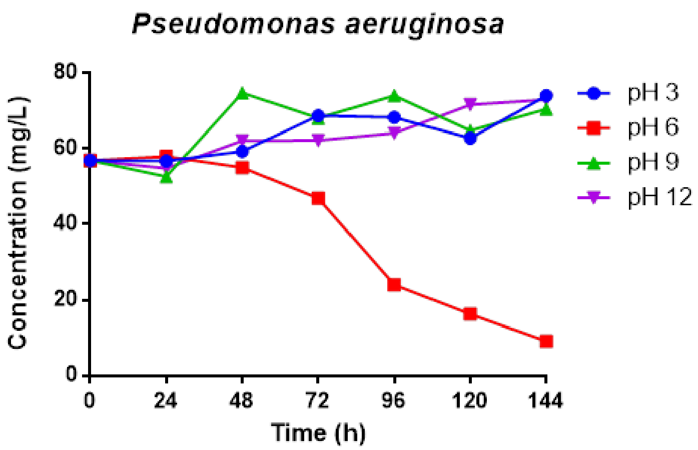

Figure 7: Changes in nitrite concentration in the wastewater in presence of the *Pseudomonas aeruginosa* at varying pH

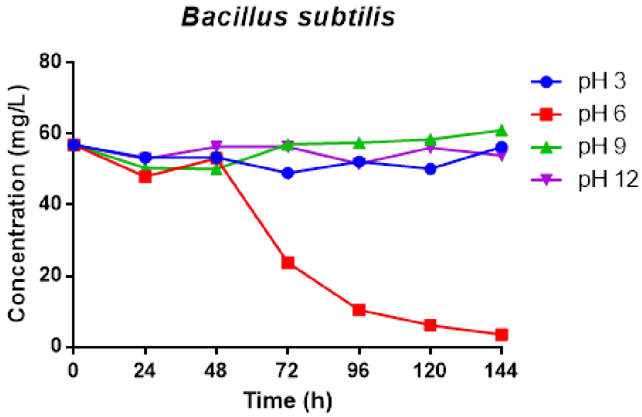

Figure 8: Changes in nitrite concentration in the wastewater in presence of the *Bacillus subtilis* at varying pH

In the carbonated feather experimental set up, no reduction in nitrite concentration was observed within the first 96 h of the experiment. This is irrespective of the varying nitrite concentration used. However, at 120 and 144 h, the carbonated feather was observed to show as much as 22.7% and 23.4% reduction at the lowest nitrite concentration. The carbonated feather sorbent showed no observable reduction in nitrite level at higher nitrite concentration (Fig. 9).

As shown in Figure 4.10, the raw feather was more effective in removing the nitrite at a lower concentration. This is revealed in the results obtained after 24 h in which the raw feather was able to remove about 87% of the initial nitrite concentration (15 mg/mL) at 0 h compared to 49, 18, 42 and 58% reduction recorded when the nitrite concentration was at 21, 40,50 and 64 mg/mL respectively (Fig. 10).

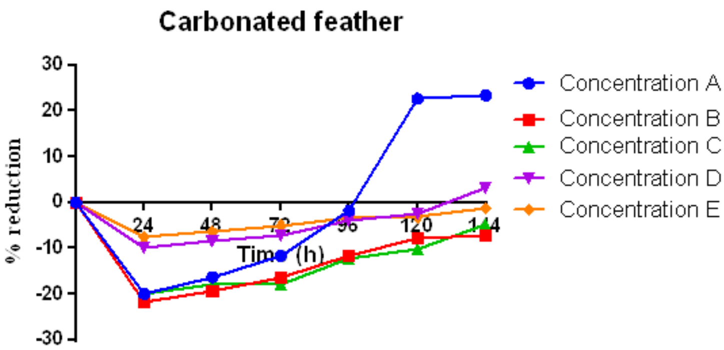

Figure 9: Changes in nitrite concentration in the wastewater in presence of the carbonated chicken feather at varying initial nitrite concentration in the wastewater

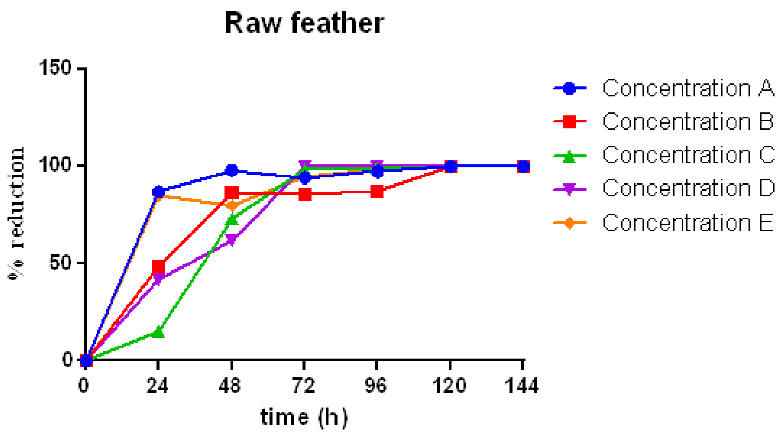

Figure 10: Changes in nitrite concentration in the wastewater in presence of the raw chicken feather at varying initial nitrite concentration in the wastewater

Pseudomonas aeruginosa was observed to show some potential nitrite reduction effect at varying nitrite concentration only after 120 h (Fig. 11). Nitrite removal in presence of the *P. aeruginosa* was also observed to be more effective when the initial nitrite concentration in the wastewater was low (Fig. 11).

As shown in Figure 12, the nitrite-reduction potential of *Bacillus subtilis* was observed at the lowest nitrite concentration (only after 96 h of treatment) and the highest nitrite concentration (from 24 h of treatment). At varying nitrite concentration within these two extremes, the carbonated feather showed no appreciable reduction capacity within the experimental period (Fig. 12).

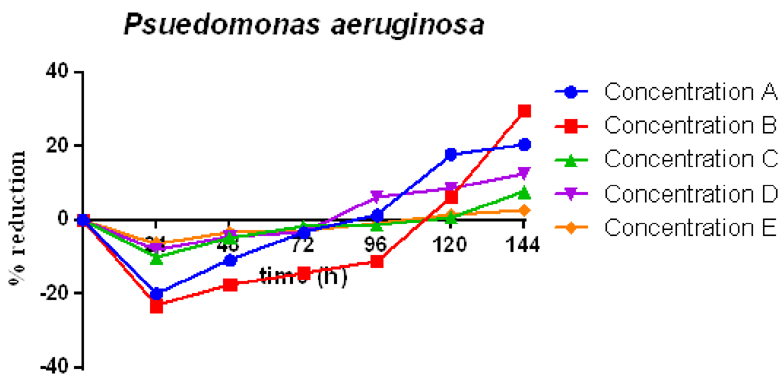

Figure 11: Changes in nitrite concentration in the wastewater in presence of *Pseudomonas aeruginosa* at varying initial nitrite concentration in the wastewater

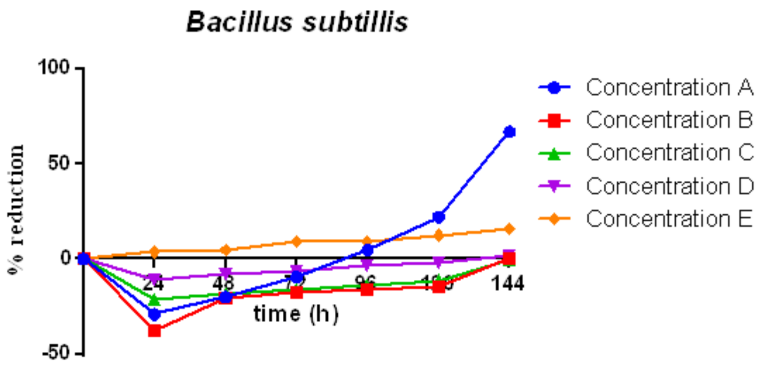

Figure 12: Changes in nitrite concentration in the wastewater in presence of the *Bacillus subtilis* at varying initial nitrite concentration in the wastewater

5. DISCUSSION

This study made use of both raw and carbonated chicken feather. Chicken feathers are known to have high tensile strength, water insolubility and stability over a wide range of pH and structural toughness due to its high efficiency, easy to handle, environment-friendly, a low-cost price compared to the conventional approaches in wastewater treatment [7]. At the different dosage of the adsorbent (2 g, 4 g, 6 g and 8 g), the rate of nitrite removal was directly proportional to the quantity of of feather used. A similar observation has been reported. A similar observation has been reported earlier [8].

In the experimental setups involving the bacterial species, the external carbon source used was sodium acetate. Sodium acetate has been reported and advocated as an ideal carbon source during biological nutrient removal studies. White and Gadd, [9], have reported sodium acetate and ethanol as effective carbon sources in nutrient removal studies. Similarly, Bharati and Kuma, [10], have indicated that the presence of sodium acetate as an external carbon source enhanced nutrient uptake from wastewater by microbial cells.

This study was carried out at 25 ºC. Biological activity has been reported to accelerate in warm temperature and slow in cool temperatures, but when the temperature is extremely hot or cold it can stop the treatment process due to this reason temperature of wastewater is regarded as an essential factor that determines the nutrient removal capabilities of test isolates and adsorbent. In this experiment, the temperature is kept constant at 25 ºC till the end of the treatment process. Similar studies have revealed optimum temperature for nutrient removal to be 30 ºC when using chicken feather for the elimination of lead in wastewater [7]. Delgadillo-Mirquez et al. [11] have reported 72 to 83% nitrogen removal from wastewater with a mixed microalgae and bacteria culture in a study carried out at 25 °C, the average total nitrogen and phosphorus removal extents ranged from 72 to 83% and 100% respectively.

However, nitrite removal was observed to increase with increasing concentration of the chicken feather fibre. A similar observation has been reported by earlier workres. In a study with banana and orange peels, nutrient removal rate was observed to increase with time as the concentration of the peels increased [12]. Time is an important factor to be considered for the wastewater treatment process. When using the feather fibre, significant nitrite reduction was mostly observed in this study between 120 and 144 h of incubation. Similar observation has been reported earlier [13].

In the present study, at the different concentrations of the initial inoculums of the bacterial species used for investigation, no significant decreases were observed in nitrite level at the end of incubation. This trend was irrespective of the test isolate used for the inoculation. Earlier investigator showed that an increase in inoculum size leads to an increase in the nutrient uptake by the bacteria test isolates [14].

At the respective pH investigated, optimum nitrite removal was observed at pH 6 in presence of the test isolates. At pH 3, 9 and pH 12, no significant reduction was observed in nitrite level throughout the period of incubation. In the case of the adsorbents, the optimum pH for nitrite removal was observed at pH 6, 9, and 12 while no significant nitrate reduction was at pH 3. Soumya et al. [15]) indicated optimum pH for nitrate and phosphate removal to range from 5 to 9 when he used seagrass for from aqueous solutions. Hydrogen ion concentration has been considered to be one of the most crucial controlling factors in adsorption processes. pH is an important variable during nutrient removal. This is because it affects the stability of the wastewater treatment process [15].

In setups with the test bacterial species, the study revealed no decrease in nitrite levels when the initial concentration of nitrite in the wastewater was high. Significant increases in nitrite levels were observed throughout the period of incubation. A similar trend has been reported elsewhere [16, 17].

6. CONCLUSION

The findings of the study revealed nitrite removal in presence of the raw chicken feather fibre was dependent on the quantity of feather fibre used. The carbonated feather was not observed to adsorbed nitrite from the wastewater under the experimental conditions investigated. Nitrite removal in presence of the test bacterial species was dependent on pH and the initial conecntration of nitrite in the media.

Generally, this study confirmed the effectiveness of raw chicken feather as a suitable natural sorbent for nitrite removal from wastewater. The study was able to provide valuable information on the role of chicken featheribre and the test bacterial species in the removal of nitrite from wastewater.

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