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Comparison of phosphate removal from wastewater by Chicken feather Hydrolysate and Agarose-Immobilized Microbial Cells

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ABSTRACT

The discharge of untreated wastewater with excessive nutrient concentration is known to cause eutrophication in receiving water bodies. This study was aimed at comparing the effectiveness of chicken feather hydrolysate and the immobilized cell of *Pseudomonas aeruginosa* and *Aspergillus brasilliense* in the removal of phosphate from wastewater. The study was carried out at varying conditions, which were the effect of initial inoculum size of the immobilized cells used or the initial quantity of the feather hydrolysate, effect of pH, effect of static and agitated conditions and the effect of batch cycle in the removal of phosphate from wastewater in presence of the feather hydrolysate or immobilized cells. The results obtained revealed a direct relationship between phosphate adsorption and quantity of hydrolysate used. In presence of the feather hydrolysate, pH and incubation condition (agitated or static) did not show any visible effect in phosphate adsorption from the wastewater unlike in presence of the immobilized cells, where remarkable phosphate removal was observed under agitated conditions and at pH 8 and 10. In addition, phosphate decrease in the wastewater in presence of the feather hydrolysates or immobilized cells was observed only during the first and second batch cycles. Comparatively, phosphate decrease in the wastewater was significantly higher in presence of the feather hydrolysates than the immobilized cells. The findings of this study could help in the development of low cost, ecofriendly and effective materials for application in wastewater treatment systems.

Key words : *Phosphate, Hydrolysate and Agarose-immobilized microbial cells*

Introduction

Due to increased discharge of detergents and excreta from homes, domestic wastewater is the major source of phosphorous in municipal effluents. The concentration of phosphorous in domestic waste water is influenced by urbanization, industrial development and lifestyle such as nutrition (Abdel-Raouf *et al*., 2012). Although phosphate is one of the major nutrients required by living organism to perform many physiological functions, when present in high concentrations, it is regarded as a pollutant. The phosphate content of clean water is between 0.01 and 0.03 mg/L. Concentrations of phosphate

higher than 0.3 mg/L indicates water pollution by fertilizer, sewage, industrial waste or detergents (Nguyen, 2015).

The primary effect of excessive concentration of nutrients from agriculture or sewage effluent is eutrophication. Eutrophication, also known as hyperthrophication is a complex process that occurs both in fresh and marine waters where excessive development of certain types of algae results as a response to the presence of excess nutrients mainly phosphate as a result of detergents, fertilizers or sewage pollution (Jung *et al*., 2014).

The conventional approaches in wastewater treatment embrace chemical precipitation and filtration, chemical reaction, chemistry treatment, reverse diffusion, evaporation, natural action, and activity. However, most accessible approaches for the removal of nutrients might demonstrate cost-efficient and technical drawbacks like inflated capital and dealing prices, high sensitivity to operating conditions, energy utilization or sludge formation (Ruzhitskaya and Gogina, 2017). Although chemicals processes have been effectively used in wastewater treatment, due to their several drawbacks, biological methods are being advocated (Akpor *et al*., 2015).

Poultry waste, within the variety of feather and blood, are of importance in biotechnology thanks to their high super molecule content. Chicken feathers are reported to possess high executable strength, quality of water, its steadiness over a variety of pH scale, structural sturdiness thanks to its high proficiency, eco friendliness and low cost (de la Rosa *et al.*, 2008; Akpor *et al*., 2018). Hence the utilization of chicken feathers for the treatment of wastewater could serve as a cost-efficient technique.

There has been redoubled interest within the use of microbial immobilized cells for wastewater treatment. The foremost benefits of the utilization of immobilized cells over standard mechanical system is its robust resistance to nephrotoxic chemicals. The utilization of immobilized cells is indicated to be lot expedient than immobilized enzymes in waste product treatment as they're natural insoluble carriers of protein activities that permits for substantial price decrease their continuous use (Shawky *et al.*, 2015; Chen and Worrall, 2016).

Considering these circumstances, it is necessary to develop effective, low-cost, and atmosphere friendly ways for water treatment. This study therefore seeks to compare the potential effectiveness of chicken feather hydrolysates for phosphate removal from wastewater with microbial immobilized cells.

Materials and Methods

Preparation of immobilized cells

The microbial species used for the study were *Aspergillus brasiliense* (ATCC 16404) and *Pseudomonas aeruginosa*(ATCC 9027). Before usage, the isolates were cultured as broth cultures before subculturing in sabauroud dextroseand nutrient agar plates, *Aspergillus brasiliense* and *Pseudomonas aeruginosa*, respectively.

For immobilization, 4 % of the agarose solutions were used. To a 200 mL of the sterile agar or agarose solution (cooled to 45 ^{o}C), 30 mL of the overnight broth culture of the respective isolate was added and agitated at 120 rpm for 3 min. The respective agarose-microbe solution was poured in approximately 20 mL quantity into petri dishes and allowed to solidify. Using a sterile cork borer, holes were bored in solidified agarose-microbe to obtain uniform beads (1 cm in diameter). The beads were stored in sterile glass bottles and stored at 4 ^{o}C, until when needed.

Preparation of feather hydrolysates

For preparation of feather hydrolysates, raw chicken feathers were collected from Landmark University Commercial Farm Omu-Aran Kwara state. The feathers were first washed with water and detergent and rinsed properly to remove every trace of detergents. The washed feathers were then sundried for 2 d.

For hydrolysis, to 200 g of the dried feather, 1 L of 0.8 M NaOH was added, mixed and allowed to hydrolyze at room temperature for 24 h. After hydrolysis, the mixture was sieved to separate any unhydrolyzed component of the feathers. To recover the hydrolysate from the filtrate, 10 % of the known acid (H_2SO_4 or HNO_3) was added gradually until the hydrolysate is formed, which was recovered from solution by filtration with a muslin cloth. The filtrate (referred to as hydrolysate) was air-dried to constant weight and stored in clean plastic bottles at room temperature.

Wastewater preparation

The wastewater used for the study was obtained from the Landmark University Commercial Farm

Omu-Aran Kwara state. Before use, the wastewater was first filtered using a muslin cloth and sterilized with an autoclave at 121 °C for 15 min at 15 psi. For studies with the immobilized cells, the wastewater was supplemented with glucose (5g/L), sodium nitrate (0.5g/L) and $MgSO_4$ (0.5 g/L). Prior to sterilization, the filtered wastewater was dispensed in 200mLquantities into 250 mL conical flasks.

Experimental procedure

To a 250 mL capacity conical flask, containing 200mL of sterile wastewater,a known quantity of the respective immobilized beads or feather hydrolysate was added and incubated in an orbital shaker (STUART SI500) at 120 rpm. Just before inoculation with the feather hydrolysate or immobilized cells and every 24 h, for 120 h duration, aliquot wastewater sample was withdrawn from each flask for the estimation of phosphate using standard procedures (APHA, 2012).

For the study, the experimental setup was carried out in four categories. The first category investigated the effect of varying concentrations of feather hydrolysate or immobilized cells on phosphate removal in the wastewater. The varying quantities of feather hydrolysates used were 5 g, 10 g, 15 g and 20 g while the number of immobilized beads used for investigation were 40, 60, 80 and 100 beads.

The second experimental category varied the pH of the wastewater before inoculation with the feather hydrolysate or immobilized cells. The pH studied were pH 6, 8, 10. The third category varied the incubation condition, wither agitated or static setup. The final category was the effect of batch cycle on phosphate removal in presence of the feather hydrolysates or immobilized cells. Three batch cycles were carried out for the study, with each batch running for 120 h, after which the respective feather hydrolysates or immobilized cells were recovered and used for inoculation of the next batch.

For each of the experiment setups, a control, which was not inoculated was run alongside with the inoculated setup. All experimental analyses were carried out in triplicate.

Statistical analysis

All data were analyzed using the SPSS statistical software (version 19.0) at probability level of 0.05. Test of significance of means was determined using the One-Way Analysis of Variance (ANOVA) test.

Results

Effect of hydrolysate quantity and initial inoculum size

In presence of the H_2SO_4 feather hydrolysate, phosphate concentration in the wastewater showed remarkable decrease at the end of incubation. A lowest phosphate decrease to 4.47 mg/L was observed after 72 h of incubation when 15 g of the hydrolysate was used for inoculation. At the end of incubation, phosphate level in the wastewater varied from 83.82 mg/L to 28 mg/L, from 88.83 mg/L to 57.63 mg/L, from 87.85 mg/L to 56.16 mg/L and from 88.83 mg/L to 55.67 mg/L, when 5 g, 10 g, 20 g of feather hydrolysate were used, respectively (Fig.1). Generally, significant increase in phosphate adsorption was observed with increase in hydrolysate quantity used ($p= 0.05$).

When the HNO_3 feather hydrolysate was used for inoculation, a decrease was observed in the concentration of phosphate in wastewater after 120 h incubation period with varying concentrations from 81.47 mg/L to 79.52 mg/L, from 81.60 mg/L to 47.68 mg/L, from 81.75 mg/L to 20.07 mg/L and from 81.42 mg/L to 13.64 mg/L in setups containing 5 g, 10 g, 15 g, 20 g, feather hydrolysate, respec-

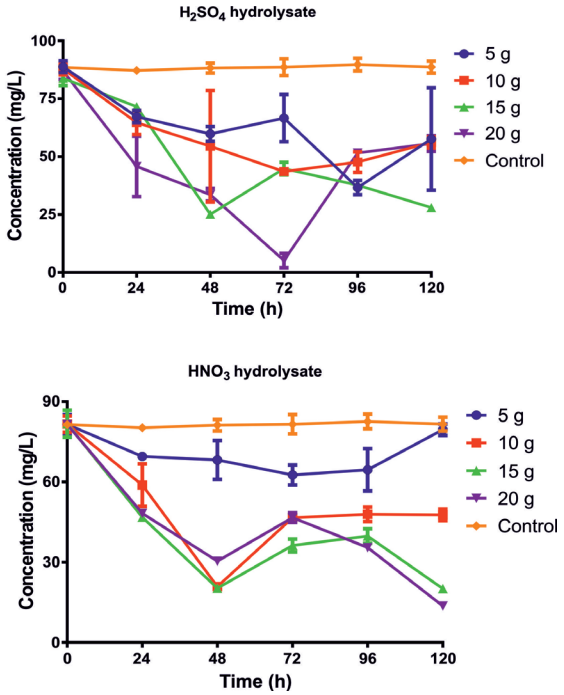

Fig. 1. Effect of the hydrolysate quantities on phosphate concentration in the wastewater

tively (Fig 4.2). Significant increase in phosphate adsorption was observed with increasing hydrolysate quantity (p= 0.05).

In the presence of quantities of the agarose-immobilized *Pseudomonas aeruginosa*, phosphate concentration in the wastewater throughout the incubation period was observed to show only minute decreases. Remarkable decrease in phosphate level in the wastewater was only observed when 60 beads were used as initial inoculum, showing a decrease in phosphate level from an initial concentration of 81.60 mg/L to 47.68 mg/L (Fig. 2). There was no significant difference between phosphate decrease in setup with the different inoculum concentrations (p= 0.05)

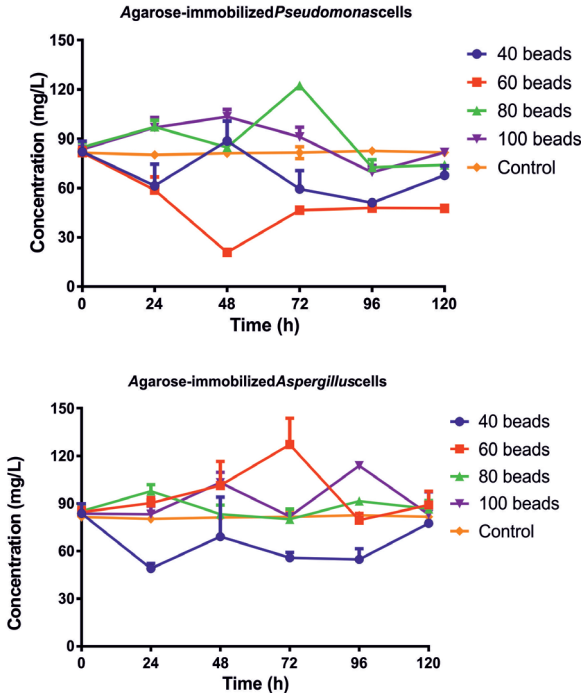

Fig. 2. Effect of initial inoculum of the microbial-immobilized cells on phosphate removal from the wastewater.

In the presence of agarose-immobilized *Aspergillus brasiliense*, remarkable decrease in phosphate level in the wastewater was only observed after 24 h incubation when the initial inoculum size was 40 beads, showing a decrease from 83.67 mg/L to 49.02 mg/L, after which there was an increase with time. At the end of the incubation period no remarkable decrease in phosphate level was observed with time. This trend was irrespective of the initial quantity of bead used for inoculation (Fig. 2). Phosphate decrease was not however observed to significantly differ between setups with the respective inoculum concentrations (p= 0.05).

Generally, highest phosphate decreases of 66.6 % and 75.45 % were recorded when 15 g of the H_2SO_4 and HNO_3 hydrolysates were used for the study, respectively. In presence of the immobilized cells, highest removal was recorded when 40 beads were used for inoculation (Table 2).

Effect of static and agitated incubation conditions

In both categories of incubation setups, notable decreases in phosphate concentration were observed in presence of the hydrolysates throughout the incubation period. After the 120 h incubation period, phosphate concentration showed reduction from 90.87 mg/L to 56.68 mg/L and from 91.05 to 58.09 mg/L, in presence of the H_2SO_4 and HNO_3 hydrolysates, respectively. For the static setup, at the expiration of incubation, phosphate levels in the wastewater showed variation from 95.80 mg/L to 48.21 mg/L and from 97.30 mg/L to 64.29 mg/L, in presence of the H_2SO_4 and HNO_3 hydrolysates, respectively (Fig. 3). There was no significant difference between phosphate concentration in agitated and static setups (p= 0.05).

In the agitated setup, phosphate level in the wastewater in presence of the immobilized cells varied at the end of the 120 h incubation period

Table 1. % Change in phosphate concentration in the wastewater at the end of the 120 h incubation in presence of the feather hydrolysates and immobilized cells

Feather Hydrolysates			Microbial-immobilized cells		
Hydrolysate weight	H_2SO_4	HNO_3	No of beads	*Pseudomonas*	*Aspergillus*
5 g	11.0	1.5	40	18.6	7.4
10 g	36.7	41.6	60	8.1	-5.3
15 g	66.6	75.4	80	12.6	-1.8
20 g	36.0	25.7	100	2.2	0.6
Control	0.87	4.2	control	-2.0	0.87

from 93.09 mg/L to 86.89 mg/L and from 90.63 mg/L to 73.99 mg/L, in presence of the *Pseudomonas* and *Aspergillus* immobilized cells, respectively. For the static setup, only minute decreases in the concentration of phosphate was observed at the end of the incubation period (Fig. 4). There was no observed significant difference between phosphate concentration in agitated and static setups in presence of the immobilized cells (p= 0.05).

At the different incubation condition, remarkable decreases in the phosphate concentration were observed both in the static and agitated setups. Higher decreases at the expiration of incubation was however observed to be in the static setup. In the case of the immobilized cells, remarkable decrease at the end of incubation was only observed in the agitated setups (Table 2).

Effect of pH

When the H_2SO_4 feather hydrolysate was used for inoculation, considerable decrease in phosphate level was observed at pH 6, pH 8, pH 10 with highest decrease observed at pH 8 (Fig 4.7). After the expiration of the incubation period, phosphate levels in the wastewater varied from 115.76 mg/L to 104.53 mg/L, from 108.44 mg/L to 56.97 mg/L and from 100.6 mg/L to 75.07 mg/L at pH 6, pH 8, pH 10, respectively (Fig. 45). Although highest removal was observed at pH 8, this was not observed to be significant (p= 0.05).

In the case of the setup that contained the HNO_3 feather hydrolysate was used for inoculation, considerable decrease in phosphate level was observed at pH 6, pH 8, pH 10 with highest decrease ob-

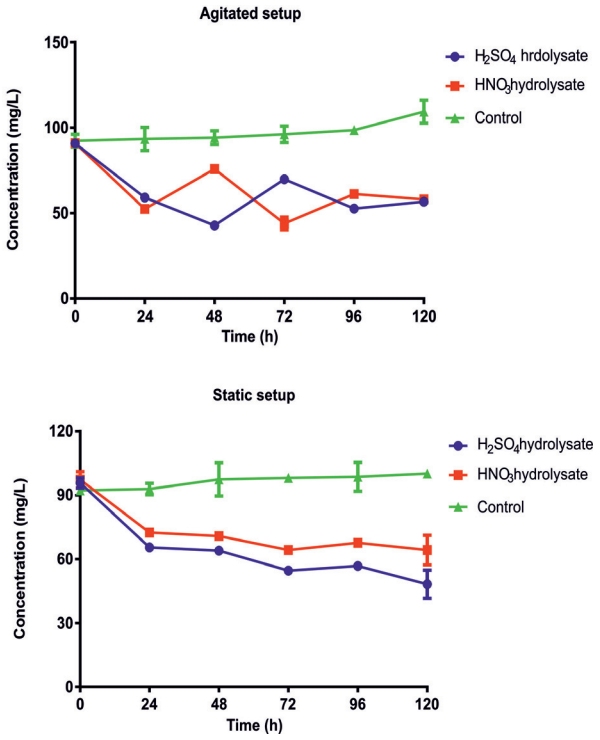

Fig. 3. Effect of agitated and static condition on phosphate adsorption from the wastewater in presence of feather hydrolysates

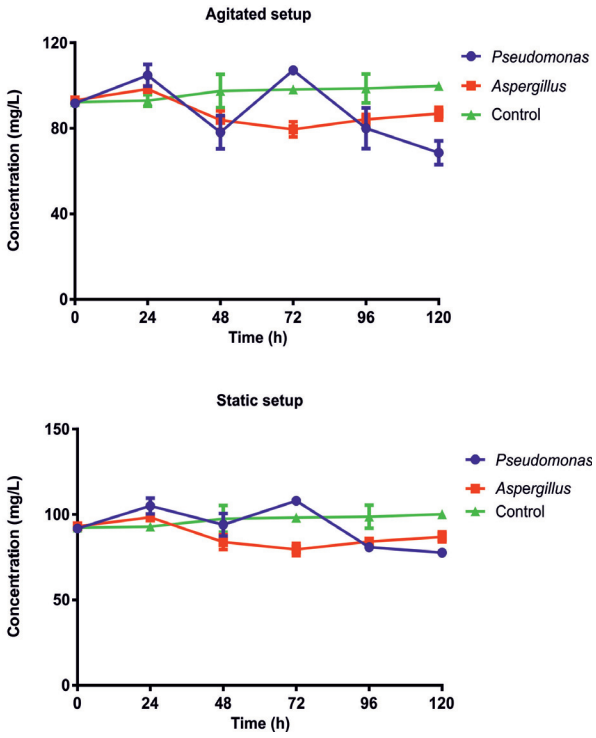

Fig. 4. Effect of agitated and static condition on phosphate removal from the wastewater in presence of immobilized cells

Table 2. % Change in phosphate concentration in the wastewater at the end of incubation at the different incubation conditions

Incubation condition	Feather hydrolysates		Immobilized cells		Control
	H_2SO_4	HNO_3	*Pseudomonas*	*Aspergillus*	Control
Agitated setup	37.6	32.6	25.1	22.8	-1.28
Static Setup	49.4	33.9	15.4	6.7	-8.5

served at pH 6. Phosphate levels at the end of incubation varied from 109.72 mg/L to 54.53 mg/L, from 105.44 mg/L to 89.65 mg/L and from 99.30 mg/L to 73.39 mg/L at pH 6, pH 8, pH 10, respectively (Fig 5). There was no observed significant difference between phosphate removal at the different pH investigated (p= 0.05).

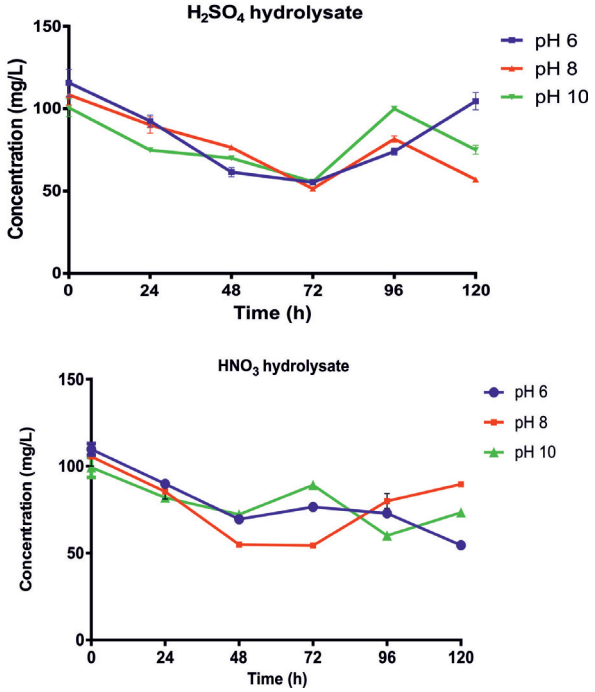

Fig. 5. Effect of pH on phosphate concentration in the wastewater in presence of the feather hydrolysates

For the setup involving the agarose-immobilized *Pseudomonas aeruginosa*, reduction in the concentration of phosphate was observed to be highest at pH 10. Only minute reduction in phosphate concentration was observed at pH 8 and 10. At the end of incubation, phosphate level in the wastewater decreased from 99.63 to 98.49 mg/L, from 97.34 to 75.80 mg/L and from 92.27 to 55.26 mg/L, at pH 6, 8 and 10, respectively (Fig. 6). Phosphate concentration did not show any significance at the difference pH studied (p= 0.05).

When the agarose-immobilized *Aspergillus brasiliense* cells were used for inoculation, only minute decreases in phosphate concentration in the wastewater were observed at the end of incubation. This observation was irrespective of the pH investigated. At the expiration of incubation, phosphate concentration varied from 98.32 mg/L to 80.45 mg/L, from 95.06 mg/L to 80.21 mg/L and from 96.18 mg/L to 82.77 mg/L, at pH 6, 8 and 10, respectively (Fig. 6). No significant difference in phosphate levels were observed at the different pH investigated (p= 0.05).

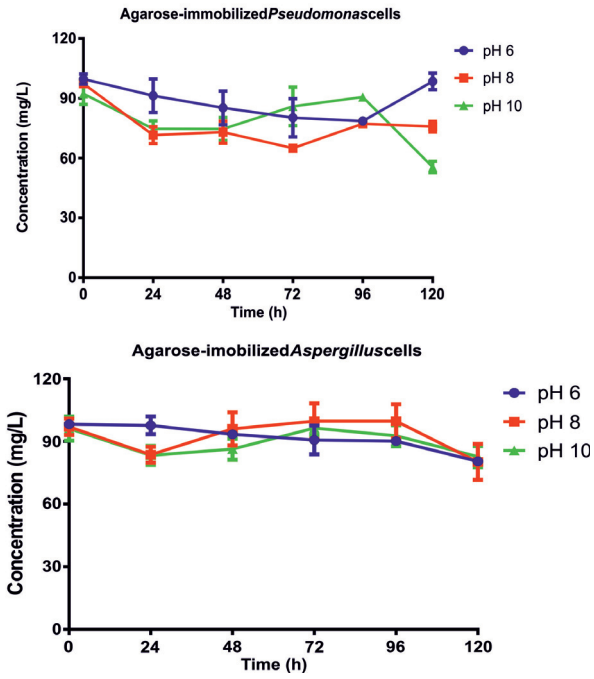

Fig. 6. Effect of pH on phosphate concentration in the wastewater in presence of the immobilizedcells

The highest phosphate decrease at the end of incubation was observed at pH 6 and 8 in presence of the HNO_3 and H_2SO_4 hydrolysates, respectively. Generally, phosphate removal in the wastewater at the end of incubation was highest at pH 6 and 10 in presence of *Aspergillus* and *Pseudomonas* immobi-

Table 3. % Change in phosphate concentration in the wastewater at the respective pH after the 120 h incubation period in presence of the hydrolysates and immobilized cells

pH	Feather hydrolysates		Immobilized cells		Control
	H_2SO_4	HNO_3	*Pseudomonas*	*Aspergillus*	
6	9.7	50.3	1.1	18.18	-1.1
8	47.5	15.0	22.1	15.62	-3.8
10	25.4	26.1	39.8	13.94	-1.5

lized cells, respectively (Table 3).

Effect of batch cycle

When the H_2SO_4 feather hydrolysate was used for inoculation, the highest decrease in phosphate concentration at the end of incubation was observed when used in the first batch. No considerable decrease in phosphate concentration in the wastewater was observed at the end of the second and third batch. Decrease in phosphate concentration in the wastewater was observed to be significantly higher in the first and second batches than the third experimental batch (p= 0.05).

For the HNO_3 hydrolysate setup, decrease in phosphate concentration was observed to be highest second batch. Increase in phosphate concentration was observed in the third batch while only minute decrease was observed in the first batch (Fig. 7). Decreases in phosphate concentration were lower with increase in experimental batch (p= 0.05).

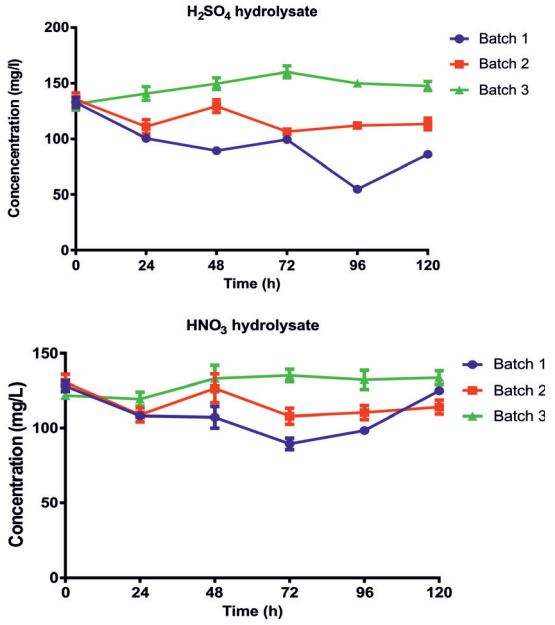

Fig. 7. Effect of batch cycle on phosphate concentration in wastewater in presence of the feather hydrolysates

Phosphate concentration in the wastewater in presence of the *Pseudomonas*-immobilized cells revealed remarkable decrease from 105.78 mg/L to 86.07 mg/L during the first batch. Slight decrease in phosphate concentration from 106.79 mg/L to 97.19 mg/L was observed during the second batch.When the *Aspergillus*-immobilized *Aspergillus* cells were used for inoculation, phosphate decrease from 28.73 mg/L to 119.24 mg/L and from 126.45 mg/L to 112 mg/L, for batch 1 and 2, respectively was observed at the end of the 120 h incubation period. No decrease in phosphate concentration was observed at the end of batch 3 (Fig. 8). In presence of the immobilized cells, no significant difference was observed in phosphate concentration between the first and second batches (p= 0.05).

Highest phosphate removal of 35.1% and 18.0%,

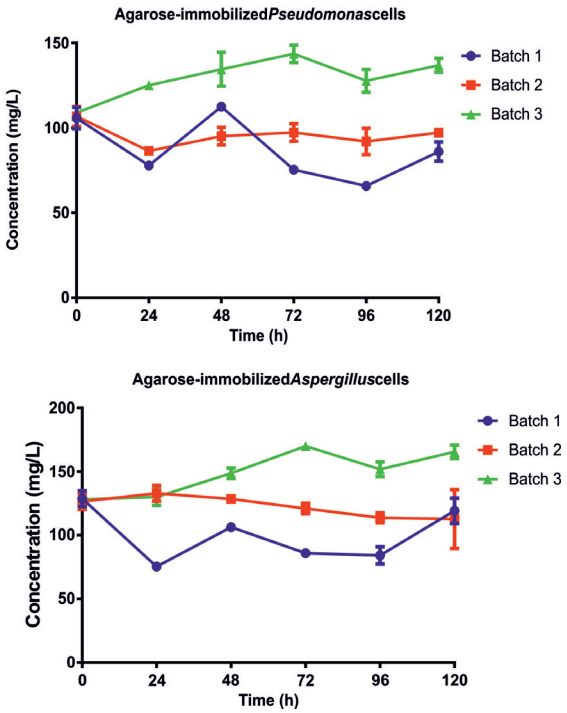

Fig. 8. Effect of batch cycle on phosphate concentration in wastewater in presence of the immobilized cells

Table 4. % Change in phosphate concentration in the wastewater after the 120 h incubation period in presence of the feather hydrolysates and immobilized cells

Batch	Feather hydrolysates		Immobilized cells		Control
	H_2SO_4	HNO_3	*Pseudomonas*	*Aspergillus*	
Batch 1	35.1	2.4	18.6	7.4	-2.0
Batch 2	16.4	12.7	22.8	10.9	1.0
Batch 3	-12.4	-9.9	-25.7	-29.1	-0.2

were during the first and second batch in presence of the H_2SO_4 and HNO_3 hydrolysates, respectively. In presence of the immobilized cells, highest removal at the end of the period of incubation was however observed during the second experimental batch (Table 4).

Discussion

The feather hydrolysates in this study obtained through alkaline hydrolysis and acid precipitation. Earlier investigators have reported alkaline hydrolysis to be effective in the breakdown of waste that contains keratin and collagen (Akpor *et al.,* 2019). This choice of chicken feather hydrolysate and microbial immobilized cells for this study was deliberate. Chicken feathers are said to have reported to have high workable strength, insoluble in water, steady over a range of pH, structurally durable, ecofriendly and cheap (Akpor *et al.,* 2018). Similarly, immobilized microbial cells are indicated to greater biosorption ability than dead bacterial cells. Some advantages that immobilized biomass offer over free cells include better reusability, high biomass loading and minimal clogging in continuous flow systems (Rani *et al.,*2010).

Although phosphate adsorption occurred in presence of the different hydrolysate doses that were used, highest adsorption was observed with increased dose up to an extent. A similar trend has been reported by earlier workers. When reporting on the effect of adsorbent dose on phosphate adsorption, Jung *et al.,* (2014) indicated increased adsorption with increase in adsorbent dose up to a certain point. Similarly, Ramakrishnaiah and Vismitha, (2012), observed in a related study, increased percentage removal increased with increasing adsorbent dose, which was attributed to likely increase in total available surface area of the adsorbent particles.

This study revealed no relationship between the quantity of immobilized cells used and the extent of phosphate removal in the wastewater. In most of the cases, highest removal rate was obtained with lowest inoculum size. The findings of the study corroborated the observation of Ruzhitskaya and Gogina (2017), who in a study on the effect of algal density on wastewater nutrient removal it was indicated that the cell concentration did not have effect on phosphate removal. The result of this study however negated the report of Manach, (2004) who indicated a direct relationship between biomass concentration -and the capacity of phosphate removal.

When the experiment was performed under agitated or static conditions, the study revealed no significant difference in phosphate adsorption at the different conditions. This observation was irrespective of the hydrolysate used. In a study on dye adsorption, optimum condition was adsorption to be in shaking conditions (Sukhvinder and Amandeep, 2015). It is hypothesized that optimization of incubation period is an essential factor in biological nutrient removal systems (Purewal *et al.,* 2015). In presence of the immobilized cells however, phosphate removal was observed to be significantly higher in agitated than static setups. This trend was irrespective of the immobilized cells used for inoculation. In a study carried out on the production of amylase by *Pseudomonas aeruginosa,* it was reported that incubation period and condition play an important role in the production of amylase enzyme. Maximum enzyme production has been reported to be higher in agitated than static conditions (Lall *et al.,* 2015).

In the present study, remarkable phosphate adsorption in presence of the respective hydrolysates was observed at the different pH investigated. A pH of 9 has been recorded to be the optimum for phosphate adsorption in a related study (Ali *et al.,* 2014). In a study that employed hydrogel as sorbent for the treatment of wastewater, adsorption capacity of the sorbent was observed to be within a wide range of 4 to 9 (Feng *et al*., 2012). A related study with alum sludge, a pH of 7.5 was reported to be optimum for phosphate adsorption (Krishnaswamy *et al*., 2009). In presence of the immobilized cells, significantly higher phosphate removal was observed at pH 6. A similar observation has been reported by earlier researchers (Hena *et al.,* 2015). It is indicated that enzyme activity is retarded at pH that is outside the range of 6-8 (Moon *et al.,* 2017). In a study of pH effect and temperature on phosphorus removal properties of layered hydroxide synthesized from zinc acetate, it was reported that at pH 6, significantly pure single-phase zinc acetates were recovered and was irrespective of the temperature of aging with 99 % of phosphate removal obtained (Saqib *et al*., 2016). In another study, during investigation of the effects of pH, molar ratios and pre-treatment on phosphorus recovery through struvite crystallization from effluent of anaerobically digested swine wastewater, optimum pH for phosphate removal

was observed to be between 8.0 and 9.0 (Robalds *et al.,* 2016).

At the different experimental batches investigated, phosphate adsorption in presence of the hydrolysates was observed to be higher during the second batch. Only minute or no decrease in phosphate was observed in the third batch. This observation was irrespective of the hydrolysate used. Some investigators have reported that longer incubation conditions enhanced nutrient adsorption (Lall *et al*., 2015). In the case of microbial immobilized cells, phosphate removal was observed to highest in the second experimental batch. This observation corroborates the observation of Carla *et al*., (2015), who indicated, nutrient removal efficiency was observed to increase with increasing retention time.

Conclusion

The findings from the study revealed no direct relationship between inoculum concentration of the immobilized cells and phosphate removal. In presence of the feather hydrolysates, phosphate decrease in the wastewater had a direct relationship to quantity of hydrolysate used.

In presence of the immobilized cells, decrease in phosphate concentration in the wastewater was pH dependent but was irrespective of pH in presence of the respective hydrolysates. Also, no significant variation in phosphate decrease in the wastewater was observed under static and agitated conditions of incubation in presence of the feather hydrolysates. However, in presence of the immobilized cells, remarkable decrease in phosphate concentration was only observed in the agitated conditions, with minute or no removal observed in static conditions. The study further revealed phosphate decrease in wastewater was dependent on the batch cycle that the feather hydrolysate or immobilized cells was used.

Comparatively, phosphate decreases in presence of the hydrolysates were significantly higher than decreases in presence of the immobilized cells. The result from this study indicates that chicken feather hydrolysate has higher adsorption properties compared to microbial immobilized cells. The findings of this study could help in the development of low cost, ecofriendly and effective adsorbents that can be implemented in wastewater treatment systems.

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