BIOREMEDIATION OF ABATTOIR WASTE–WATER USING ALGAE AND IMMOBILIZED CELLS OF *ASPERGILIUS NIGER*

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Abstract

The role of algae and immobilized Aspergilus niger cells in waste stabilization ponds for abattoir wastewater bioremediation has been investigated. Using two sets of three constructed facultative ponds containing different concentrations of immobilized A.niger cells (0, 50, and 100 beads / l, respectively for each set of ponds), the removal of BOD, COD, nitrates, phosphates, turbidity, total solids and colour was measured over a 16-day period. One set of ponds was placed under illumination while the other set of ponds were placed in a dark room to suppress the growth of algae in order to investigate the synergy or otherwise between algae and the fungus in the ponds. All parameters were measured in accordance with the procedures of Standard Methods for the Examination of Water and Wastewater. The results revealed that Aspergilus niger removed 69.4-77.8 % of BOD and 72.1-78.7 % of COD after 16 days. When combined with algae, BOD, COD, phosphates, nitrates and turbidity removals were 88.5-92.3 %, 97.4-97.7 %, 87.0-87.6 %, 52.9-54.4 % and 80.2-81.5 %, respectively. The study showed that the algae and the Aspergilus cells act synergistically in bioremediation of abattoir wastewater.

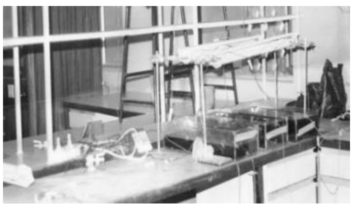
Keywords: *Bioremediation, algae, A. niger, waste stabilization pond, BOD.*

INTRODUCTION

Bioremediation has recently established itself as a promising and rapidly changing technology that has emerged within the environmental remediation industry. Bioremediation is a technology that uses biological systems to solve pollution problems and it has been considered to be far more cost-effective than traditional cleaning technologies with possible savings of 65-85 % (Indu, 2006). Biological treatments remove organic compounds and pathogens from the effluent using microorganisms. As a microbial process, it requires the provision of nutrients among other factors or requirements for micro organisms to break down pollutants into simpler compounds such as CO_2, H_2O, and mineral salts. Several studies exist on the use of fungi and other microorganisms for bio-treatment of raw wastewaters, they include the works reported or reviewed by Andleeb *et al*. (2010), Akthar & Mohan (1995); Coulibaly *et. al*. (2003); Feijoo & Lema (1995); Kapoor & Viraraghavan, 1995, 1998; Kapoor *et al*. (1999); Meyer & Wallis (1997); Palma *et al*., (1999); Pasti-Grigsby *et al*. (1992); Sumathi & Phatak (1999); Tatarko & Bumpus (1998); Tereshina *et. al*. (1999); Wong & Yu (1999); Yasmin *et al*. (2009); Zhou (1999) to mention a few.

Waste stabilization ponds are shallow ponds in which sewage effluents are stored. Natural biological action takes place during the storage period with resulting oxidation and reduction of organic matter. Oxygen necessary for biological activity is gotten from re-aeration and photosynthesis. In the presence of light, algae utilize the end products of bacterial decomposition of organic matter and liberate oxygen (Mara, 1984).

The activity in the WSPs is a complex symbiosis of bacteria and algae, which stabilizes the waste and reduces pathogens. The algae produce oxygen during photosynthesis by utilizing carbon dioxide and solar energy derived from sun light. The bacteria utilize oxygen for the biological process to convert the organic content of the effluent to more stable and less offensive forms and release carbon dioxide. This interdependence



of algae and bacteria is known as mutualistic or algae – bacteria symbiosis. They can be used alone or in combination with other treatment processes. Studies have also shown that apart from providing oxygen for bacterial growth, algae can play a direct role in the degradation of some wastewaters **(**Liu and Liu, 1992). *Aspergillus niger* is a member of the genus *Aspergillus* which includes a set of fungi that are generally ubiquitous in nature and are geographically widely distributed and commonly found as a saprophyte growing on dead leaves, stored grains, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil (Arora *et al*., 1992). This study was conducted to evaluate the effectiveness of immobilized cells of *Aspergillus niger* in the purification of polluted effluents of abattoirs and to investigate the role played by algae (synergistic or antagonistic) in the bioremediation process.

MATERIALS AND METHODS

Sample Collection and Analysis

Samples were collected from the outlet point of the gutters running an abattoir Zaria. The wastewater samples were collected in two 25 L jerry cans and taken to the Environmental Engineering Laboratory at Ahmadu Bello University, Zaria, Nigeria for analysis. The fungi *Aspergilus niger* were cultured using Sabouraud glucose agar as growth media. After 7 days, the cells were harvested and immobilized in sodium alginate gel using the method described by Lee (1992). **Fig. 1:** Experimental setup showing the three facultative ponds with the florescent tubes fixed above them to provide light to the ponds in order to facilitate the growth of algae.

Immobilization of Cells

The *Aspergilli* cells that had been grown in the Fuji bottles was mixed with 30 ml of the tween 80 solution by dispensing the tween 80 into the Fuji bottles containing the grown cells , then covering the bottles and shaking the bottles vigorously to form a homogenous mixture. The homogenous mixture was poured from the Fuji bottles into a beaker. The sodium alginate solution was then added to the homogenous mixture in the beaker. The cell alginate solution was then pumped through a 5 ml injection syringe and fed drop-wise into a flask containing sterilized 0.12M $CaCl_2$. The droplets instantly reacted with $CaCl_2$ to form spherical beads. The beads were left in the solution for 1 h to ensure that precipitation reaction reaches completion.

Experimental Procedure

The abattoir wastewater sample collected was poured into the anaerobic laboratory pond and the whole surface covered with black polythene bag after which the pond was covered with a big wooden plank. The sample was left in the pond for 12 days for anaerobic digestion. The effluent from the anaerobic pond was introduced by gravity into the three facultative ponds, which were all connected to the anaerobic pond via rubber tubes till they were all filled up. Two florescent tubes fixed above the liquid surface provided light for the facultative ponds as shown in Fig. 2. The immobilized cells were then introduced into the facultative ponds, which were labeled A, B, and C with respective concentrations levels of 0 beads/litre, 50 beads/litre and 100 beads/litre. Samples were taken initially (at the start of experiment), and periodically after 2-3 days interval from the facultative ponds. All the samples were analyzed for BOD, COD, nitrates, sulphates, phosphates, turbidity, total solids and colour using standard methods (APHA, 1998). The experiment was repeated in a darkroom to suppress the growth of algae in order to study the effect of algae on the bioremediation process.

Calculation of Percentage Removal

The percentage removals were obtained by using the following equation:

$$\frac{A-B}{A} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where

A = initial concentration

B = concentration at time t

RESULTS AND DISCUSSION

The values of the BOD, COD, nitrates, sulphates, phosphates, turbidity, total solids and colour after 2, and 9 days were recorded as shown in Tables 1 and 2 and the percentage reductions in BOD, COD, nitrates, sulphates, phosphates, turbidity, total solids and colour after 16 days were recorded as shown in Table 3 for both the algae inclusive and algae exclusive experiments. The physico-chemical quality of the wastewater after 2 days and 9 days are as shown in Tables 1 and 2, respectively, it is shown that the wastewater was better polished at higher retention time of 9 days. The table 3 shows that after 16 days treatment, the algae inclusive run had better BOD removals of 69.2 %, 92.3 % and 88.3 % for the control, pond A and pond B respectively; than the algae exclusive run which had 58.3 %, 77.8 % and 69.4 % removals in the three ponds respectively. The ability of *A. niger* to bioremediate the wastewater is clearly shown by the performance of the ponds which contain the fungi with respect to the control pond. The table also shows that algae plays a complimentary or synergistic role to *A. niger* in BOD removal in the ponds, this finding is in agreement with previous studies (Liu & Liu, 1992) in which it was found apart from producing oxygen during photosynthesis, certain algae can also degrade pollutants in wastewater.

Table 1: Physico-chemical quality of wastewater after 2 days treatment

Parameters	Algae inclusive Experiment			Algae Exclusive Experiment		
	Control	Pond B	Pond A	Control	Pond B	Pond A
BOD(mg/l)	1200	1200	1300	1600	1500	1600
COD(mg/l)	7595	4472	4004	8769	8365	8548
Nitrates(mg/l)	125	129	122	90	80	80
Sulphates (mg/l)	1440	1427	1400	1083	1056	1167
Phosphates (mg/l)	195	244	195	387	356	342
Turbidity (NTU)	396	385	410	410	430	400
Total solids(mg/l)	3080	3350	3240	4300	4120	4270
Colour(hazen)	40	40	40	50	50	50
pH	7.40	6.80	7.00	7.73	7.77	7.81

Table 2: Physico-chemical quality of wastewater after 9 days treatment

Parameters	Algae inclusive Experiment			Algae Exclusive Experiment		
	Control	Pond B	Pond A	Control	Pond B	Pond A
BOD(mg/l)	600	700	500	950	1100	700
COD(mg/l)	3528	1703	2520	6310	5104	4672
Nitrates(mg/l)	91	91	85	80	70	60
Sulphates (mg/l)	1309	1286	1210	856	793	807
Phosphates (mg/l)	122	98	98	195	168	171
Turbidity (NTU)	214	220	220	298	289	276
Total solids(mg/l)	1520	1260	1170	2360	2430	2470
Colour(hazen)	30	30	30	30	30	30
pH	7.50	5.80	7.40	8.31	8.25	8.09

The COD removal as shown in Table 3 also followed the same trend as that of the BOD. The percentage removals of COD in the algae inclusive run were 75.5 %, 97.7 % and 97.4 % for the control, pond A and pond B respectively while the algae exclusive runs had 52.2 %, 72.1 % and 78.7 % removals in the three ponds, respectively. Again, the bioremediation ability of *A.niger* is evident in the performance of the ponds with the fungi as compared to the control pond without it. The higher percentage removals by the algae inclusive run suggests that algae are involved the COD removal from the wastewater. The removal of nitrates from the wastewater by the algae inclusive ponds were 48.5 %, 54.4 % and 52.9 % for the control, pond A and pond B respectively while the algae exclusive run had 40.0 %, 50.0 % and 50.0 % removals for each of the ponds respectively. These percentage reductions are all within the same range and thus show that *A. niger* and algae did not play significant roles in nitrates removal as the ponds with and without *A. niger* and algae did not differ much in percentage removals. High percentage removals of phosphates were observed in all the ponds, this may be due to the fact that phosphorous is essential to the growth of algae and other biological organisms (Grady *et al*., 1999; Tchobanoglous *et al.,* 2003). The algae inclusive ponds had 84.0 %, 87.0 % and 87.6 % removals, respectively for the control pond; pond A and pond B while in the algae exclusive experiment, pond A and B both had 74.9 % removal while the control pond had 68.9 %. These values are lower than those of the ponds with algae and this suggests that algae play a key role in removal of phosphates. This may be due to the fact that algae make use of phosphorous compounds by converting them to further algal mass (Metcalf & Eddy 2003).

Table 3: Percentage reductions of the physico-chemical quality of the wastewater after 16 days of treatment

Parameters	Algae inclusive Experiment			Algae Exclusive Experiment		
	Control	Pond B	Pond A	Control	Pond B	Pond A
BOD(mg/l)	69.2	92.3	88.5	58.3	77.8	69.4
COD(mg/l)	75.5	97.7	97.4	52.2	72.1	78.7
Nitrates(mg/l)	48.5	54.4	52.9	40.0	50.0	50.0
Sulphates (mg/l)	18.2	26.0	31.0	48.8	56.9	53.2
Phosphates (mg/l)	84.0	87.0	87.6	68.7	74.9	74.9
Turbidity (NTU)	61.5	80.2	81.5	52.3	54.8	56.0
Total solids(mg/l)	71.1	75.4	75.4	57.8	71.1	62.0
Colour(hazen)	57.1	57.1	57.1	57.1	57.1	57.1

The percentage reductions for sulphate as shown in Table 3 indicate that the sulphate removals were low in the algae inclusive ponds although ponds A and B with *A. niger* were slightly higher than the control pond. Sulphate removals in the algae exclusive ponds were observed to be higher than those of the algae inclusive ponds. This suggests that algae may have some inhibitory effect on sulphate adsorption or some non-photosynthetic microorganisms that can adsorb sulphates may be present in the pond. Turbidity removal in the algae exclusive ponds were 61.5 %, 80.2 % and 81.5 % for the control, pond A and pond B, respectively implying that the ponds with *A. niger* had better turbidity removal than the control pond. Comparison of the algae inclusive run with that of algae exclusive run which had 57.8 %, 71.1 % and 62.0 % for the control, pond A and pond B, respectively shows that the algae must have played a role in turbidity removal since the ponds without algae did not remove turbidity as much as the ponds with it.

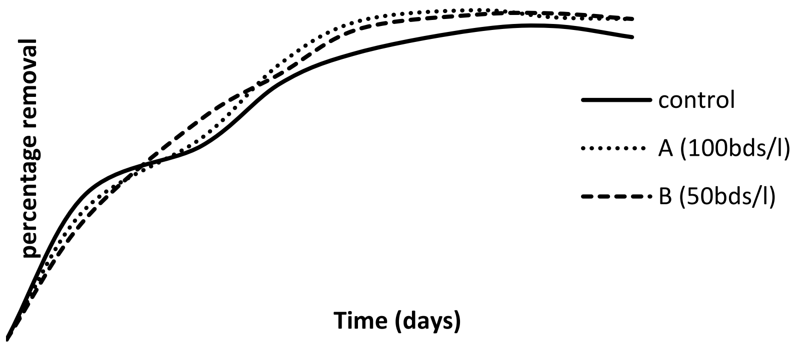


Fig. 2: Total solids removal in the algae inclusive run

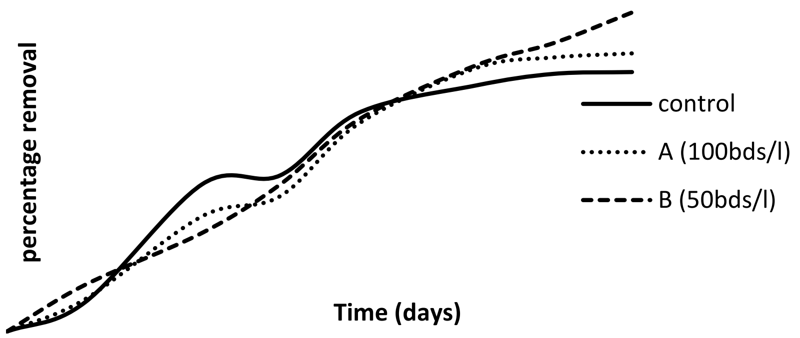


Fig. 3: Total solids removal in the algae exclusive run

The removal of total solids was higher in the algae inclusive ponds than the algae exclusive ponds. However, there was increase in the total solids contents of the algae inclusive ponds between 12 to 16 days retention time which slightly decreased the percentage removal as shown in fig. 2. This increase can be attributed to the significant growth of algae on the facultative ponds. The increase was not observed in the algae exclusive ponds (Fig. 3) as algae did not grow on the ponds; even though the total solids removal was still lower than the algae inclusive ponds.

CONCLUSION

The findings of this study showed that algae plays synergistic role with *A.niger* in the removal of BOD, COD, phosphates and turbidity. Nitrates removals were not influenced by the presence of *A. niger* and algae while higher sulphate values were observed in ponds without algae. Total solids were observed to increase in ponds with algae at 12-16 days retention time due to algal growth.

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