

Evaluation of some Species of *Aspergillus* for their Biodegradation capability to Degrade Silk Industry Effluent of Reservoir Khajua Lake at Mubarakpur, Azamgarh, (U.P.)

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The present study was carried out to observe the biodegrading character of *Aspergillus niger*, *Aspergillus aculatus* and *Aspergillus flavus* on the hydrological parameters pH, alkalinity, DO, BOD, COD and hardness. Dyeing processes in silk industries release dyes and other chemicals in nature. Biodegradation of such effluent from Khajua lake at Mubarakpur was conducted with respect to the above parameters. These parameters were estimated at 50%, 75% and 100% concentrations of the effluent at three different time intervals 24 hours, 96 hours and 120 hours.

The results of the experiment indicate that the standard values recommended by WHO for these parameters were approximated after 120 hours of the experiment by the treatment with the microbes under study. The biodegradation due to the microbes was found maximum at 50% concentration of the silk industry effluent while it was minimum at 100% concentration. The use of *Aspergillus niger*, *Aspergillus aculatus* and *Aspergillus flavus* was found satisfactory for purification of silk industry effluent.

Keywords: *Aspergillus*, biodegradation, silk industry effluent, Khajua lake.

Textile and silk industries release a large amount of untreated waste water which is finally discharged into adjacent water bodies. This waste water causes soil and ground water pollution. Silk industry at Mubarakpur is a major industry of eastern U.P. The entire liquid waste from this industry is blown away without any pretreatment into drainage lines which are ultimately connected with Khajua lake. The water contains waste chemicals, high values of pH, BOD, COD and alkalinity etc. than the standard values prescribed by the pollution control board and WHO. It is possible to degrade the pollutant in the waste water by employing microorganisms, as biological processes work faster in tropical conditions¹⁸.

Biological methods are much effective in reduction of pollution load of effluent. Activated sludge process and its modification are the commonly used biological methods for the removal

of colour and organic matter from dye house wastes.

Palleria & Chamber (1995), observed the effect of white rot fungus *Tremetes versicolor* on decolourization and found 56.77% continuous decolourization and 41.69 AOX reduction with retention time of 24 hours in cell free system.

Heinfling *et al.* (1997), observed the effect of fungus *Tremetes versicolor*, *Bjerkandera ajusta* and *Phanerochaete chrysosporium* on azo dye orange 96 and two phthalacyanine dyes reactive blue 15 and reactive blue 38 and found that these fungus were able to decolorize the above dyes.

Rodriguez *et al.* (1997), studied the effect of ascomycetes fungus *Chrysonilia sitophila* on biodegradation of carpet industry effluent and observed that lignin was biodegraded by this fungus.

Mittal *et al.* (1997), observed the ability to absorb cations by saprophytic fungus *Fomotopsis*

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carnea and found that the fungus was able to absorb dye cations viz. orlamerned blue and yellow.

Heinfling *et al.* (1998), observed that *Bjerkandera adjusta* produced manganese peroxide which transform industrial dye.

Wong & Yu (1999), studied the decolourization ability of fungus *Tremete versicolor* on three synthetic dyes anthraquinone, azo and indigo and observed that enzyme laccase, an extracellular oxidase, was involved in decolourization.

Ponting & Vrijmoed (2000), noticed that white rot basidiomycetes fungi *Phanerochaete chrysosporium* have ability to decolorize several azo triphenyl methane and heterocyclic polymeric dyes after 14 days. Perumal *et al.* (2000), observed another white rot fungus *Gonoderma lucidium* and found that the fungus have the ability to decolorize paper mill effluent.

For the purpose of microbial examination, water and soil samples were collected monthly for a period of one year from the two sampling stations. The samples were collected from Khajua lake at Mubarakpur, Azamgarh (U.P.)

Identification of microorganisms present in culture were carried out in the laboratory itself. Then after these culture were sent to MIT Chandigarh for identification and confirmation of species.

Exponentially grown culture were harvested by centrifugation, washed with sterilized double distilled water and homogenized with sterilized neutral glass beads. A known inoculum of such homogenized suspension was then incubated in a liquid culture medium supplemented with graded concentration of silk industry effluent. The effects of *Aspergillus niger*, *Aspergillus aculatus* and *Aspergillus flavus* at 50%, 75% and 100% concentrations of the effluent were assessed at 24, 96 and 120 hours time interval by analyzing various changes in pH, alkalinity, DO, BOD, COD and hardness.

All the results obtained during the experiment were statistically analysed by using the theory of analysis of variance for two way classification.

The pH values of the effluent at 50%, 75% and 100% concentrations at zero hour were recorded as 8.1, 8.6 and 8.9 respectively. Due to

the effect of the three species of *Aspergillus*, each concentration of the effluent showed a gradual decrease in its pH value with the passage of time. When treated with *Aspergillus niger*, after 120 hours of the experiment the pH values at the above stated three concentrations were 6.9, 7.1 and 7.5 respectively. When the effluent was treated with *Aspergillus aculatus*, the pH values were recorded as 7.0, 7.2 and 7.3 at 50%, 75% and 100% concentrations respectively after 120 hours. Treatment with *Aspergillus flavus* showed the pH values as 7.1, 7.2 and 7.7 at the above stated three concentrations after 120 hours of the experiment. These results show that the pH value of the effluent at 50% concentration after 120 hours approximated the pH value of clear water when treated with the three species of *Aspergillus*.

The alkalinity of the effluent at 50%, 75% and 100% concentrations at zero hour were 740, 760 and 775 mg/L respectively. Treatment with the three species of *Aspergillus* brought gradual decrease in alkalinity at all the three concentrations. When treated with *Aspergillus niger*, the alkalinity was reduced to 298, 322 and 350 mg/L at 50%, 75% and 100% concentrations respectively after 120 hours. The minimum alkalinity was recorded as 298 mg/L at 50% concentration after 120 hours.

The application of *Aspergillus aculatus* reduced the alkalinity values to 290, 306 and 320 mg/L at 50%, 75% and 100% concentrations respectively after 120 hours of the treatment. The minimum alkalinity value was recorded as 290 mg/L at 50% concentration after 120 hours.

After 120 hours of the treatment, *Aspergillus flavus* reduced the alkalinity values to 280, 320 and 341 mg/L at the above stated three concentrations respectively. The minimum value of alkalinity was 280 mg/L which was found after 120 hours at 50% concentration.

The DO values of the effluent at 50%, 75% and 100% concentrations at zero hour were recorded as 4.9, 3.7 and 1.6 mg/L respectively. The application of the three species of *Aspergillus* affected the DO values of the effluent showing a gradual decrease during 24 hours and then after an increase was observed at all the three concentrations. After 120 hours of treatment with *Aspergillus niger*, the DO values of the effluent at 50%, 75% and 100% concentrations were 8.2, 6.9

and 2.3 mg/L respectively. 8.2 mg/L was the highest DO value which was recorded after 120 hours at 50% concentration. When treated with *Aspergillus aculatus*, the DO values of the effluent at 50%, 75% and 100% concentrations were found as 9.7, 6.8 and 2.5 mg/L respectively after 120 hours. The maximum DO value was 9.7 mg/L which was observed after 120 hours at 50% concentration. Due to the effect of *Aspergillus flavus*, the DO values of the effluent at 50%, 75% and 100% concentrations were observed as 8.9, 7.1 and 2.8 mg/L respectively after 120 hours of the treatment. The maximum DO value in this case was 8.9 mg/L which was attained at 50% concentration after 120 hours.

The BOD values of the sample effluent at 50%, 75% and 100% concentrations at zero hour were 215, 261 and 344 mg/L respectively. The application of the three species of *Aspergillus* cause gradual decrease in BOD values of the effluent at all the three concentrations during 120 hours of the experiment. When treated with *Aspergillus niger*, the BOD values at these three concentrations were found as 119, 136 and 187 mg/L after 120 hours. The minimum value of BOD was 119 mg/L which was recorded at 50% concentration after 120 hours.

The application of *Aspergillus aculatus* reduced the BOD values to 105, 121, 183 mg/L at 50%, 75% and 100% concentrations respectively after 120 hours. The minimum BOD value attained was 105 mg/L at 50% concentration after 120 hours.

After 120 hours the BOD values were found as 98, 115 and 195 mg/L at 50%, 75% and 100% concentrations respectively when the effluent was treated with *Aspergillus flavus*. The minimum value of BOD in this case was recorded at 50% concentration after 120 hours.

The COD values of silk industry effluent at 50%, 75% and 100% concentrations at zero hour were 467, 692 and 899 mg/L respectively. Gradual decrease in COD values were observed at all the three concentrations when treated with *Aspergillus* species. After 120 hours of treatment with *Aspergillus niger*, the COD values of the effluent at 50%, 75% and 100% concentrations were recorded as 209, 289 and 398 mg/L respectively. The minimum COD value was found as 209 mg/L after 120 hours at 50% concentration.

When treated with *Aspergillus aculatus*, the COD values of the effluent at 50%, 75% and 100% concentrations were found as 147, 199 and 363 mg/L respectively after 120 hours. The minimum COD value was 147 mg/L which was observed after 120 hours at 50% concentration.

Due to the effect of *Aspergillus flavus*, the COD values of the effluent at 50%, 75% and 100% concentrations were observed as 158, 212 and 311 mg/L respectively after 120 hours of the treatment. The minimum COD value was noted after 120 hours at 50% concentration.

The hardness of the effluent at 50%, 75% and 100% concentrations at zero hour were 389, 561 and 701 mg/L respectively. When the effluent was treated with *Aspergillus* species, hardness decreased gradually at all the three concentrations through out the period of experiment. After 120 hours of treatment with *Aspergillus niger*, the hardness of the effluent became 171, 240 and 286 mg/L at 50%, 75% and 100% concentrations respectively. The minimum hardness value was recorded at 50% concentration after 120 hours.

When treated with *Aspergillus aculatus*, the hardness of the effluent at 50%, 75% and 100% concentrations were found as 143, 207 and 277 mg/L respectively after 120 hours. The minimum value of hardness was 143 mg/L which was observed after 120 hours at 50% concentration.

Due to the effect of *Aspergillus flavus*, the hardness of the effluent at 50%, 75% and 100% concentrations were observed as 165, 196 & 309 mg/L respectively after 120 hours of the treatment. The minimum value of hardness was 165 mg/L which was recorded at 50% concentration after 120 hours.

The process of biodegradation reduces the pollution load of effluent as it oxidises organic and inorganic contents of the effluent. In the present study, the three species of *Aspergillus* have demonstrated their importance in silk industry effluent treatment.

The effect of *Aspergillus niger*, *Aspergillus aculatus* and *Aspergillus flavus* at 50%, 75% and 100% concentrations of silk industry effluent were assessed at 24, 96 and 120 hours time interval by analysing various changes in physicochemical characteristics of the effluent viz. pH, alkalinity, DO, BOD, COD and hardness.

Table 1. Analysis of the effect of three species of *Aspergillus* with respect to pH.

Conc % / Time hr	50			75			100			Mean		
	A	B	C	A	B	C	A	B	C	A	B	C
0	8.1	8.1	8.1	8.6	8.6	8.6	8.9	8.9	8.9	8.533	8.533	8.533
24	7.2	7.5	7.6	7.7	7.8	7.9	8.1	8.6	8.4	7.666	7.970	7.970
96	7.1	7.2	7.3	7.5	7.3	7.4	7.8	8.4	8.1	7.466	7.630	7.600
120	6.9	7.0	7.1	7.1	7.2	7.2	7.5	7.3	7.7	7.166	7.370	7.330
Mean	7.32	7.45	7.52	7.72	7.72	7.77	8.07	8.45	8.27			
F (Time)→A—155.28*, B—26.52*, C—68.06*,				CD(Time)→A—0.163, B—0.33, C—0.218								
F (Conc)→A—84.59*, B—37.32*, C—48.99*,				CD(Conc)→A—0.141, B—0.29, C—0.188								
A - <i>Aspergillus niger</i> ,			B - <i>Aspergillus aculatus</i> ,			C - <i>Aspergillus flavus</i>						

Table 2. Analysis of the effect of three species of *Aspergillus* with respect to Alkalinity.

Conc % / Time hr	50			75			100			Mean		
	A	B	C	A	B	C	A	B	C	A	B	C
0	740	740	740	760	760	760	775	775	775	758.33	758.33	758.33
24	520	518	520	625	620	630	735	690	731	626.67	609.33	627.00
96	310	327	320	375	390	390	515	480	511	400.00	399.00	407.00
120	298	290	280	322	306	320	350	320	341	323.33	305.33	313.67
Mean	467	468.7	465	520.5	519	525	593.7	566.25	589.5			
F (Time)→A—49.59*, B—85.19*, C—59.37*,				CD(Time)→A—98.83, B—76.77, C—91.2								
F (Conc)→A—6.63*, B—6.45*, C—7.46*,				CD (Conc)→A—85.59, B—66.48, C—78.98								
A - <i>Aspergillus niger</i> ,			B - <i>Aspergillus aculatus</i> ,			C - <i>Aspergillus flavus</i>						

Table 3. Analysis of the effect of three species of *Aspergillus* with respect to DO.

Conc % / Time hr	50			75			100			Mean		
	A	B	C	A	B	C	A	B	C	A	B	C
0	4.9	4.9	4.9	3.7	3.7	3.7	1.6	1.6	1.6	3.4	3.4	3.4
24	3.7	3.1	3.8	3.1	2.9	2.5	1.4	0.9	1.2	2.733	2.3	2.5
96	7.1	6.8	7.2	5.2	5.1	6.3	1.8	1.3	2.2	4.7	4.4	5.23
120	8.2	9.7	8.9	6.9	6.8	7.1	2.3	2.5	2.8	5.8	6.33	6.27
Mean	5.97	6.125	6.2	4.72	4.62	4.9	1.77	1.57	1.95			
F (Time)→A—6.91*, B—6.92*, C—10.8*,				CD(Time)→A—1.8, B—2.26, C—1.8								
F (Conc)→A—22.96*, B—16.89*, C—23.35*,				CD(Conc)→A—1.56, B—1.95, C—1.56								
A - <i>Aspergillus niger</i> ,			B - <i>Aspergillus aculatus</i> ,			C - <i>Aspergillus flavus</i>						

Table 4. Analysis of the effect of three species of *Aspergillus* with respect to BOD.

Conc % / Time hr	50			75			100			Mean		
	A	B	C	A	B	C	A	B	C	A	B	C
0	215	215	215	261	261	261	344	344	344	273.33	273.33	273.33
24	169	181	169	186	197	198	261	237	257	205.33	205.0	208.0
96	131	126	141	149	145	165	211	197	216	163.66	156.0	174.0
120	119	105	98	136	121	115	187	183	195	147.33	136.33	136.0
Mean	158.5	156.75	155.7	183.0	181.0	184.7	250.2	240.2	253.0			

F (Time)→A—51.6*, B—43.31*, C—65.9*, CD(Time)→A—27.1, B—32.13, C—24.88
 F (Conc)→A—49.75*, B—28.6*, C—64.4*, CD(Conc)→A—23.47, B—27.83, C—21.55

A - *Aspergillus niger*, B - *Aspergillus aculatus*, C - *Aspergillus flavus*

Table 5. Analysis of the effect of three species of *Aspergillus* with respect to COD.

Conc % / Time hr	50			75			100			Mean		
	A	B	C	A	B	C	A	B	C	A	B	C
0	467	467	467	692	692	692	899	899	899	686.0	686.0	686.0
24	301	285	296	398	369	387	671	632	735	456.67	428.67	472.67
96	242	169	193	327	235	248	483	467	492	350.67	290.33	311.0
120	209	147	158	289	199	212	398	363	311	298.67	236.33	227.00
Mean	304.75	267.0	278.5	426.5	373.7	384.7	612.7	590.25	609.2			

F (Time)→A—23.74*, B—44.51*, C—20.82*, CD(Time)→A—122.11, B—104.31, C—153.47
 F (Conc)→A—25.82*, B—39.91*, C—19.37*, CD(Conc)→A—105.75, B—99.33, C—132.91

A - *Aspergillus niger*, B - *Aspergillus aculatus*, C - *Aspergillus flavus*

Table 6. Analysis of the effect of three species of *Aspergillus* with respect to hardness.

Conc % / Time hr	50			75			100			Mean		
	A	B	C	A	B	C	A	B	C	A	B	C
0	389	389	389	561	561	561	701	701	701	550.33	550.33	550.33
24	311	287	296	379	363	346	518	511	487	402.67	387.0	376.33
96	260	196	201	267	281	278	319	328	389	282.0	268.33	289.33
120	171	143	165	240	207	196	286	277	309	232.33	209.0	222.33
Mean	282.75	253.75	262.75	361.75	353	345.2	456.0	454.2	470.7			

F (Time)→A—18.35*, B—33.1*, C—37.98*, CD(Time)→A—114.62, B—90.67, C—79.8
 F (Conc)→A—9.17*, B—19.56*, C—27.57*, CD(Conc)→A—99.27, B—78.53, C—69.11

A - *Aspergillus niger*, B - *Aspergillus aculatus*, C - *Aspergillus flavus*

After 120 hours of the treatment with the said *Aspergillus* species, changes in pH values were almost same and ranged between the minimum of 6.9 at 50% concentration of effluent to a maximum of 7.7 at 100% concentration. The pH value after the treatment at 50% concentration approximated the pH value of clear water. This change was probably due to certain enzymes, operative in degradation of effluent and mineralization of organic and inorganic substances present in silk industry effluent. Similar findings have been reported by Chaudhary *et al.* (1997) & Kumar (1999).

The maximum change due to the treatment with microorganisms obtained at 50% concentration may be attributed to the percentage of concentration of the effluent as 50% concentration of silk industry effluent, which would provide better conditions for growth and multiplication of microorganisms and biodegradation. After 120 hours of the experiment no significant changes were recorded at any concentration of the effluent.

Alkalinity neutralizes the acids in natural water. The main contributors to alkalinity in natural water are HCO_3^- , CO_3^{2-} and OH^- phosphates, silicates and other bases etc. They are commonly found in the form of carbonate of sodium and bicarbonates of calcium and magnesium.

In the present study, alkalinity of silk industry effluent was observed due to discharge of carbonate, bicarbonate, caustic soda and bleaching powder used in processing²². After 120 hours of the treatment with the three species of *Aspergillus* at 50%, 75% and 100% concentrations of the effluent, the alkalinity decreased by 54.84% to 62.16%. The maximum decrease was observed at 50% concentration of the effluent. Similar findings have also been reported by Cole (1979), Kanekar & Sarnaik (1995) and Todd (1970). After the treatment with microbes, decrease in the value of alkalinity suggested active decomposition of organic matter which attributed formation of CO_2 and other secondary end products^{2,21}.

DO is an important factor in determination of water quality criteria of an aquatic system. The concentration of DO in waste water depends on physical, chemical and biological activities.

In the present study, after 120 hours of the treatment with the three *Aspergillus* species at 50% concentration of the effluent, the increase in DO was observed between 3.3 and 4.0 mg/L while it was between 3.1 and 3.4 mg/L at 75% concentration. The increase in DO values ranged between 0.7 and 1.2 mg/L at 100% concentration. The increase in DO values was maximum at 50% concentration. Similar findings have been reported by Groff & Kim (1989), Kumar (1999) and Shukla & Pandey (1991). There was no significant change in DO values after 120 hours of the experiment. Loss of DO values in the first 24 hours of treatment was noticed. It was probably due to rapid utilization of oxygen at higher rate by microorganisms. In the present study the ultimate increase in DO values due to low extent of pollution load as well as low oxidation of organic matter might be attributed to the growth of phytoplankton which enable the water to hold more oxygen^{6,10,15} concluded a similar result.

The BOD is an approximate measure of the amount of biochemically degradable organic matter present in water. The higher value of BOD indicates higher pollution load and leads to the higher consumption of DO.

During present course of study, after 120 hours of the treatment with the three species of *Aspergillus* at 50% concentration of silk industry effluent the minimum BOD value obtained was 98 mg/L while it was 115 mg/L at 75% concentration and 183 mg/L at 100% concentration. The maximum loss of BOD due to microorganisms was obtained at 50% concentration of the effluent while it was minimum at 100% concentration. It may be due to the reason that 50% concentration of the silk industry effluent provided better condition for growth and multiplication of microorganisms and biodegradation. Similar results have been reported by Kulla & Meyer (1980) and Kumar (1999).

The amount of oxygen needed for chemical oxidation of the pollutants to inorganic product is expressed by COD.

In the present study, after 120 hours of the treatment with microorganisms at 50% concentration of silk industry effluent, the COD values decreased and ranged between 147 to 209 mg/L while the COD values ranged between 199 to 289 mg/L at 75% concentration. At 100%

concentration of the effluent the COD values ranged between 311 and 398 mg/L. The decrease of COD due to biodegradation of microorganisms at 50% concentration of the effluent was maximum while it was minimum at 100% concentration. It may be due to the reason that 50% concentration of the effluent provided better condition for growth and multiplication of microorganisms and biodegradation. Similar results have been reported by Kanekar & Sarnaik (1995), Kumar (1999), Rai (2000) and Tezel *et al.* (2001).

Hardness is directly responsible for quality of water. So determination of hardness is of considerable importance in polluted waters.

During the present course of study, after 120 hours of the treatment with microorganisms at 50% concentration of the effluent, the hardness ranged between 143 and 171 mg/L while at 75% concentration the hardness ranged between 196 and 240. The range of hardness recorded at 100% concentration was 277 to 309 mg/L. The minimum value of hardness was 143 mg/L observed at 50% concentration. While 309 mg/L was the maximum value of hardness observed at 100% concentration. Higher value of hardness in silk industry effluent was observed due to discharge of carbonate, bicarbonate, caustic soda and bleaching powder etc. used in processing. The continuous decrease in hardness was due to oxidation of organic matter and mineralization of organic pollutant into secondary inorganic substances and product.

Microorganisms are responsible for biodegradation of effluent in natural system. Dyes are colouring substances. Basically all synthetic dyes are chemical compounds. These chemical compounds have the property of hydrolysis, oxidation-reduction etc. These properties are controlled by the activity of enzymes. Enzymes are operative only in living system. Microorganisms are capable to produce these enzymes. By the activity of these enzymes many intermediate and final products are formed in any system. The formation of intermediate and final products depends on availability of favorable conditions for enzyme action.

In the present study, effluent was diluted by using standard method. This might probably have facilitated the better growth of microbes in

water reservoir and favorable conditions for enzymes activity produced by microorganism at lower concentrations. The higher concentrations were proved to be toxic for better growth and activity of microorganism which have showed poor effect on the parameters undertaken.

The results obtained during the course of study were statistically analyzed and it was concluded that the results obtained were significant for both the factors- concentration and time interval.

The use of *Aspergillus niger*, *Aspergillus aculatus* and *Aspergillus flavus* was found satisfactory for purification of silk industry effluent. The biodegradation due to microbes was maximum at 50% concentration while it was minimum at 100% concentration of the silk industry effluent. At higher concentration of the effluent, the values of all the six parameters were much higher than the recommended values. After the treatment with the said species of *Aspergillus*, biodegradation was recorded at all the concentrations but optimum values of the parameters were approximated at 50% concentration of the effluent after 120 hours of the experiment. No significant improvement was recorded after 120 hours. Thus *Aspergillus niger*, *Aspergillus aculatus* and *Aspergillus flavus* may be recommended for reduction of dye toxicity in silk industry effluent fed reservoir.

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