Biodegradation of polyvinyl alcohol adsorbed on granular activated carbon

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Polyvinyl alcohol (PVA) is a water soluble polymer used as emulsifier for suspension polymerization. PVA discharged from industrial effluents poses a threat to the environment through bioaccumulation. It also has the potential to mobilize heavy metals from sediments in water streams and lakes and thus increase their bioavailability to the food chain. Adsorption of PVA onto carbon is a versatile treatment but cost prohibitive unless regenerated. This study aims at restoring the adsorptive capacity of PVA adsorbed granular activated carbon (GAC) by biodegradation catalyzed by oxidases and/or hydrolases. The biodegradation of PVA adsorbed on GAC by acclimatized microbial cultures, was carried out using specified media along with controls (without cultures). Biodegradation of aqueous solutions of PVA was studied under similar conditions using the mixed cultures. Results indicate a steady decrease in PVA concentration up to 96 hrs. Biodegradation of PVA on GAC was almost 2-3 fold higher than that of controls. The surface characterization of PVA adsorbed GAC before and after biodegradation that of an and GAC fresh sample was also carried out. Re-adsorption of PVA on bio-regenerated GAC was studied along with control. The study indicates biodegradation can be used for regaining the adsorption capacity of PVA on GAC.

Keywords: Biodegradation, polyvinyl alcohol, adsorption, re-adsorption, microbial cultures

Introduction
Polyvinyl alcohol (PVA) biodegradation is believed to be due to random chain cleavage involving two enzymes groups, oxidases and/or hydrolases. It usually works as a two step process: (i) Some bacterial species excrete extracellular PVA oxidase which results in oxidized PVA and (ii) The oxidized PVA is then spontaneously depolymerized in the 2nd step by hydrolases. The conventional biological systems are not efficient for the degradation of PVA as the PVA degrading capacity of most microbial species is very specific and limited. Carbon adsorption has proved to be one of the most versatile treatment option, particularly in treating wastewater streams having low concentrations of contaminants and in meeting stringent statutory requirements. Activated carbon (AC) based system can remove a wide variety of pollutants with very high removal efficiencies. However, the usefulness of the adsorption process lies in the operational simplicity and reuse potential of the adsorbents during long term applications. Cost associated with the use of AC are prohibitive unless the carbon can be reused.

A variety of regeneration techniques for exhausted AC6-8, especially carbon saturated with volatile organic compounds, are mostly thermal, chemical and electrochemical. Microbial degradation of the contaminants adsorbed onto the carbon is an attractive and emerging technique where the microorganisms utilize the organic substrate on the surface of AC as a source of food and energy and thus restore the AC to its former quality. Process of substrate removal includes adsorption on AC surface, adsorption on biofilm and microbiological oxidation9,10. Among the various mechanisms of biodegradation of PVA adsorbed GAC are 1) Enzymatic hydrolysis and 2) Bio degradation following desorption.

The aim of this study was to restore the adsorptive capacity of spent granular activated carbon used for adsorption of PVA by way of microbial degradation. The scope of the study included the following:

- To study the adsorption of PVA by GAC;
- To study the biodegradation of PVA by acclimatized microbes over a period of time;
- To biodegrade the PVA adsorbed on the GAC using the acclimatized cultures; and
- To study the PVA readsoption capacity of biodegraded GAC.

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Materials and Methods

Microbiological Methods

Growth of Mixed Cultures Under Sterile Conditions
For both the studies, i.e. biodegradation of PVA in solution and on GAC, the wastewater acclimatized microbes derived from an effluent treatment plant (ETP) were isolated, purified over TYG Tryptone-Yeast-Extract-Glucose agar slants and the purified bacteria were then mixed and grown to large quantities in Basal Medium (BM)11 prepared in 1 L tap water (1 g glucose, 1 g Na₂HPO₄·2H₂O, 0.6 g KH₂PO₄, 0.3 g NaCl, 0.03 g MgSO₄·7H₂O, 0.15 g TYG).

Biodegradation of PVA Adsorbed GAC and PVA in Solution
An inoculum having an OD of ~0.244 at 660 nm, of the 7 purified mixed cultures (50 ml) were then transferred under sterile conditions into the experimental flasks having ~100 ml of chemically defined media (CDM) + PVA loaded GAC (for bioregeneration) & only ~100 ml CDM (for biodegradation).

Chemical Analysis
Concentration of poly vinyl alcohol (PVA) was determined by using a modification of the colorimetric technique described by Bugada & Rudin12 where PVA in solution reacts with boric acid in the presence of potassium iodide and develops a green colour which is estimated colorimetrically at a wavelength of 690 nm. A standard curve was prepared using different concentrations of PVA versus its absorbance and the concentration (ppm) was read out using the factor derived from the standard graph.

Biodegradation of PVA Adsorbed GAC
Preparation of Granular Activated Carbon
GAC was ground and sieved such that the particle size of the GAC was <2 mm and >1 mm. It was washed with tap water, then with distilled water (DW), dried/activated at 100°C until constant weight was reached and stored in desiccator for use.

Adsorption
Batch adsorption experiments were carried out in series in 250 ml beakers containing 20 g GAC exposed to 200 ml of 625 ppm aqueous PVA solution over a period of 15 min, 30 min, 2 h, 3h & 6h. Since adsorption slowed down after 2h the period of adsorption for all experiments was stopped after 2h.

Adsorption of PVA on GAC for Biodegradation studies
Based on batch adsorption result the GAC was exposed to 625 ppm of PVA solution for 15 min, 30 min & 2 h in separate flasks and after the allotted time the GAC was separated from the solution by filtration and the filtrate was measured for PVA concentration. The difference between initial and final PVA concentration in the water was used for calculating the concentration of PVA adsorbed by the GAC for the experiments. Thus, by using 3 different loading/exposure times (15 min, 30 min & 120 min) 3 different concentrations of PVA adsorbed GAC (120 ppm, 230 ppm & 320 ppm) were obtained for biodegradation experiments.

Biodegradation of PVA Adsorbed on GAC
The PVA adsorbed GAC (~15 g) of each concentration (120 ppm, 230 ppm & 320 ppm) was transferred into three 250 ml flasks and 100 ml of sterilized CDM was added to it. Then 50 ml of acclimatized mix culture derived from ETP (50 ml) was added to each experimental flask. Controls were run using the PVA loaded GAC and sterilized distilled water. These were all shaken for periods of 3, 6, 24, 48, 96 & 144 h. At the designated hours 10 ml samples were removed, centrifuged and supernatant was estimated for PVA concentration and replenished with fresh DW. All calculations of the PVA concentration in GAC after 6 h, 48 h & 144 h of incubation are restricted to the PVA concentration in the water phase for all the studies.

Biodegradation of Aqueous Solutions of PVA
Simultaneous to biodegradation of PVA adsorbed GAC, the biodegradation of PVA in CDM was also studied at the same concentrations. This was carried out in duplicates for each PVA concentration (120, 230 & 320 ppm) in 100 ml CDM to which was added the acclimatized mix culture (50 ml), kept on shaker for 24, 48, 96 & 144 h. The PVA concentration were measured by taking 10 ml of sample from each flask, centrifuging it and taking the supernatant for measurement.

Re-adsorption of Biodegraded GAC and Control GAC
The GAC which had been treated by biodegradation and the control GAC treated by simple water wash by shaking, were re-exposed to saturated PVA solution (625 ppm) for readsorption studies over a period of 2 h and samples were analyzed at different periods (15, 30 and 120 min) of exposure.

Surface Characterizations of GAC
The surface characterizations (surface area, pore volume and pore size distribution) of fresh, control
and biodegraded GAC were carried using a Micrometrics ASAP 2020 Surface Area & Porosity Analyzer for studying changes, if any.

Results & Discussion:

The properties of the granular activated carbon (GAC) used in the experiments are as follows (Table 1).

Preliminary Adsorption Studies

In the initial adsorption study which was carried out to estimate the adsorption capacity of the GAC for PVA and to decide the period of exposure to be used for loading different concentration of PVA on the carbon, the results indicate that a sharp increase of PVA adsorption by the GAC occurs during the first two hours after which equilibrium is almost reached (Fig. 1). Hence for the biodegradation of PVA adsorbed GAC experiments a period of two hours exposure of the GAC to the aqueous phase PVA was used. Lower concentrations on GAC were also obtained by shorter exposures times.

Adsorption of PVA on GAC for biodegradation studies

Adsorption of PVA onto GAC for controls and experimental samples is shown in Figure 2 for obtaining the 3 concentrations (120, 230 & 320 ppm) tried in this study. Results show the PVA remaining in solution. The arrows indicate the period of adsorption used for obtaining the 3 different concentrations (indicated beside them) of PVA adsorbed on the GAC.

### Table 1 — Properties of GAC used in the experiments

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<th>Sr. No.</th>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
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<tr>
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<td>Appearance</td>
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<tr>
<td>6</td>
<td>Pore Volume</td>
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Microbiological Studies

Seven types of microbial colonies were predominantly seen in acclimatized cultures capable of degrading PVA (Fig. 3). These were purified on TYG slants grown to sizable quantities in BM and used for the present studies as mixed cultures.

Biodegradation of PVA adsorbed on GAC

**Biodegradation of PVA Adsorbed on GAC for 15 Minutes (120 ppm)**

Biodegradation of PVA adsorbed by GAC that was exposed to 15 minutes to PVA solution (625 ppm) where roughly an uptake of 120 ppm from solution onto the carbon was achieved (Fig. 4). Results indicate that biodegradation of the PVA on the GAC is almost two-fold that of controls, based on PVA concentration seen in the solution phase. Supporting this

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Fig. 2 — PVA removal by GAC by adsorption for Controls & experimental, calculated by PVA remaining in the solution phase.

Fig. 3 — Acclimatized cultures

Fig. 4 — Removal of PVA from GAC (120 ppm) by biodegradation (experimental), by simple water treatment (Control) and biodegradation of aqueous PVA.
observation is the trend observed with aqueous PVA biodegradation (54\% at 144 h and 66\% at 96 h incubation). Others have reported values of 34\% regeneration by microbes for phenols and chlorophenols\textsuperscript{13}. Aktas and Cecen\textsuperscript{14} reported almost 75-80 cometabolic regeneration of chlorophenol.

**Biodegradation of PVA Adsorbed on GAC for 35 Minutes (230 ppm)**

Biodegradation of PVA adsorbed by GAC that was exposed to 35 minutes to PVA solution (625 ppm) where roughly an uptake of 230 ppm from solution onto the carbon was achieved (Fig. 5). At a starting concentration of 228 ppm the biodegradation of PVA loaded GAC is almost three-fold that of controls, based on PVA concentration seen in the solution phase. The biodegradation of aqueous PVA by the microbes on the other hand is around 51\%.

**Biodegradation of PVA Adsorbed on GAC for 120 Minutes (320 ppm)**

Biodegradation of PVA adsorbed by GAC that was exposed to 120 minutes to PVA solution (625 ppm) indicating a sequestration of almost 320 ppm of the organic also shows a three-fold removal by biodegradation when compared to that exhibited by controls, based on PVA concentration seen in the solution phase (Fig. 6). Supporting this observation is the trend observed with aqueous PVA biodegradation (50\%, Fig. 7). Others have reported regeneration by microbes to the extent of 34\% & 17\% for phenols and chlorophenols\textsuperscript{13}, respectively. Aktas & Cecen\textsuperscript{14} reported almost 75-80 cometabolic regeneration of chlorophenol. An adsorbed substance is utilized through reversibility of adsorption\textsuperscript{15,16,17}. At equilibrium, an adsorbable substance is distributed between solution and AC surface. Once microorganism degrade adsorbable substance, equilibrium is disturbed and desorption occurs, which partially replenishes solution concentration. This process may be then continued until all of the substance is degraded\textsuperscript{18,20}. In the above study also the microbial degradation of PVA on GAC stabilizes after approximately 50 h, i.e. no more PVA is seen in the solution, whereas in the controls a steady desorption of the PVA into the fresh distilled water is seen and probably would have continued until all the PVA from the carbon could be desorbed.

**Biodegradation of PVA solutions**

**Biodegradation & Growth**

Biodegradation and growth (indicated by turbidity) of microorganisms is shown in Figure 7. Results indicate a steady decrease in PVA concentration till 96 h and an increase after that. At the lower exposure concentration (120 ppm) almost 60\% degradation is achieved. With the higher concentrations (230 & 320 ppm) removals of 55 to 50\% is seen at 96 h of incubation. The increase in PVA concentration seen after this is unexplainable. A similar trend is observed even with turbidity though these measurements were restricted to 0, 48 & 144 h. The initial decrease in turbidity may be more directly related to the detrimental effects of the metabolites\textsuperscript{12} of PVA.
degradation, however the 144 h increase in turbidity is more difficult to explain unless it is the metabolites themselves that may be contributing to the turbidity observed herein.

**Re-adsorption of Biodegraded GAC & Control GAC**

The PVA degraded and control GACs were again exposed to PVA solution (628 ppm) for periods of 0.25, 0.5 & 2 h as in the initial adsorption experiments. The re-adsorption capacity of the biodegraded GAC vs. control GAC in comparison to the original fresh GAC is shown in Figure 8. The PVA biodegraded carbon is able to retain 66% of PVA adsorption capacity while the controls regenerated by shaking with distilled water over a rotary shaker retained 30% of its adsorption capacity. Longer hours of biodegradation or fresh addition of cultures may help further in removal of the adsorbed PVA from the GAC. However, in the case of control GAC which had been just treated with DW, adsorption appears to have reached and equilibrium as seen in Figure 8.

Surface area characterization of the biodegraded PVA adsorbed GAC, and PVA adsorbed GAC controls after treatment with distilled water were compared to fresh GAC samples. The Biodegraded GAC was similar to fresh samples. The lowering in SA was only 6% when compared to experimental controls (10%). The pore volumes also showed that PVA adsorbed carbon showed a 17% reduction in pore volume while regenerated controls were exhibiting 31% reduction with reference to fresh GAC. This seems to support the observations made here. Thus the effective adsorption capacity (biodegraded experimentals minus distilled water washed controls) works out to 36%. This is almost similar to reported values for other compounds such as phenols and chlorophenols\(^{21,14}\). The aqueous PVA degradation (without GAC) is generally slow however in these studies ~50% degradation was achieved in a matter of 96 h. This probably is due to the fact that the microbes were acclimatized to petrochemical wastewaters which have a high loading of PVA in them. Thus, they could biodegrade the PVA adsorbed carbon successfully. Our results suggest that a process of biodegradation can be used for regaining the adsorption capacity of PVA on GAC.

Hence based on these studies one can conclude that a treatment process that includes adsorption on charcoal followed by biodegradation, such as biofiltration, may work successfully and economically in degrading the PVA in wastewaters.

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**References**