BIOREGENERATION OF POWDERED ACTIVATED CARBON (PAC) LOADED WITH AROMATIC COMPOUNDS

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Abstract—Bioregeneration of powdered activated carbon (PAC) loaded with aromatic compounds was quantitatively determined using carbon dioxide production as a measure of substrate consumption. Two types of powdered activated carbon (one chemically activated, wood-based carbon, CAI, and one thermally activated, peat-based carbon, SA4) and two aromatic compounds (o-cresol and 3-chlorobenzoic acid) in single solute batch systems were used. Interaction of EDTA, present as chelating agent in all experiments with o-cresol as substrate, and SA4 resulted in abiotic CO2 production (in absence of microorganisms). The extent of bioregeneration varied considerably, from 15 to 85% of total PAC loading, depending on type of PAC used and compound involved and, in one case, on the time of contact between PAC and the compound. The results revealed that the data corresponded with a mechanism of bioregeneration where desorption precedes biodegradation. Microorganisms reduced the dissolved compound concentration, forcing desorption and subsequent biodegradation, but were unable to influence the desorbability of the PAC-sorbed compounds. The practical applicability of the results is discussed.

Key words—activated carbon, activated sludge, adsorption, bioavailability, biodegradation, 3-chlorobenzoic acid, CO2 production, o-cresol, desorption, 2-methylphenol, wastewater treatment

INTRODUCTION

Addition of powdered activated carbon (PAC) to activated sludge (AS) wastewater treatment systems leads to an integration of the processes of sorption and biodegradation. Among the possible beneficial effects of this combination are both an enhanced stability of the process and an enhanced removal of toxic organic compounds (Sublette et al., 1982; Weber et al., 1987). Although this integrated PAC-AS system has been in operation on a practical scale for many years, the exact role of PAC in the process has not yet been elucidated.

Previous research in our laboratory on the removal of o-cresol in model wastewater treatment systems showed that PAC acted as a buffer. Shock-dosed o-cresol was temporarily adsorbed, whereas the amount of o-cresol adsorbed onto PAC decreased in the period following, concomitantly with biodegradation of the substrate. This process of bioregeneration led to an enhanced removal of shock-dosed o-cresol and to an increase in process stability in the PAC-AS system when compared to a conventional AS system (de Jonge et al., 1991).

Bioregeneration can be defined as the renewal of the adsorptive capacity of activated carbon by the action of microorganisms. Although some authors have reported on qualitative indications for the occurrence of bioregeneration (Khan et al., 1981; Chudyk and Snoeyink, 1984; Flynn et al., 1976; DeWalle and Chian, 1977; Perotti and Rodman, 1974), hardly any attempts have been made to assess the process of bioregeneration quantitatively. Still the occurrence of bioregeneration will lead to a renewal of adsorptive potential, a higher stability of the system and a prolonged duration of service by the PAC. Bioregeneration will reduce the PAC dosage required; knowledge of the process is therefore of economic as well as of environmental importance.

The mechanism of bioregeneration is not fully understood either (Sublette et al., 1982). Perotti and Rodman (1974) contend that exo-enzymes are employed in the consumption of sorbed compound. Schultz and Keinath (1984) indicate that sorbed compounds are utilized through the reversibility of adsorption. But from their experiments it is not clear if and how microorganisms might influence desorption, since the substrate they used, phenol, adsorbed in a fully reversible manner onto the PAC used.

The aim of this study is to determine quantitatively the extent of bioregeneration, as it occurs in the PAC-AS system. It will be investigated whether bioregeneration varies with the PAC species and/or compound involved and, if so, why. The data on bioregeneration will be compared with data on desorbability (de Jonge et al., 1996).
Based on previous research on sorption processes, two types of powdered activated carbon and two aromatic model compounds (o-cresol and 3-chlorobenzoic acid) have been used. In this way, measurements have been made on four PAC/compound combinations, encompassing a wide variety in adsorbability and desorbability (de Jonge et al., 1996).

**MATERIALS AND METHODS**

**Model compounds**

The compounds used were o-cresol (2-methylphenol) and 3-chlorobenzoic acid (3-CB), and were purchased from Merck (Darmstadt, Germany) in the purest form available.

**Powdered activated carbons**

Two types of powdered activated carbons were used in this study: CA1, a chemically activated carbon made from wood, and SA4, a thermally (steam) activated, peat-based PAC (Norit N.V., Amersfoort, The Netherlands). Several PAC characteristics are presented elsewhere (de Jonge et al., 1996). The PAC was washed with demineralized water, oven dried at 110°C and then stored in a sealed container prior to use.

**Mineral salts solutions**

All experiments with o-cresol were conducted in a solution of mineral salts according to Evans et al. (1970), modified as described by de Jonge et al. (1991). EDTA (ethylene diamine tetraacetic acid) was used as chelating agent. The solution was phosphate buffered (50 mM) at pH 7.0 ± 0.1. For all experiments with 3-CB a phosphate buffered (pH 7.0 ± 0.1) mineral salts solution as described by de Jonge et al. (1996) was used.

**Microorganisms**

For the experiments with o-cresol, an aerobic enrichment culture obtained from activated sludge derived from an industrial wastewater treatment plant (Tankcleaning Rotterdam, Rotterdam, The Netherlands) was used. For experiments on the biodegradation of 3-CB, *Pseudomonas* B13, kindly supplied by the Department of Microbiology, Agricultural University, Wageningen, The Netherlands, was used. Microorganisms were maintained in continuous-flow gas lift reactors with a working volume of 550 ml, as described by de Jonge et al. (1991). The following conditions were applied: dilution rate $D = 0.1 \text{ h}^{-1}$, $T = 30^\circ \text{C}$, pH 7.0 ± 0.1, airflow 201 h$^{-1}$, 3-CB influent concentration 300 mg l$^{-1}$ (1.92 mM), o-cresol influent concentration 200 mg l$^{-1}$ (1.85 mM). Microorganisms were grown aerobically with either 3-CB or o-cresol as sole carbon and energy source, in the same mineral salts solutions as used in the bioregeneration batch experiments.

**PAC loading**

PAC was loaded by adding a weighed amount of PAC to 250-ml screw-capped Erlenmeyer flasks containing mineral salts solutions with a known concentration of sorbate. Incubations were made for various contact times. After the appropriate contact time the batch was centrifuged (20 min at 14,000 g) and the PAC pellet used for a bioregeneration experiment. More details are given by de Jonge et al. (1996).

**Bioregeneration experiments**

Bioregeneration was determined by measuring CO$_2$ production in a batch culture containing adapted biomass, mineral medium and a known amount of centrifuged, preloaded PAC. A small quantity of fluid from the loading batch was introduced with the pellet into the bioregenera-

**RESULTS AND DISCUSSION**

**Bioregeneration measurement**

To assess the biodegradation of sorbed compound, several parameters, such as the protein content (as a measure of the biomass concentration) or the loading of the PAC with substrate (a direct measure of PAC regeneration) can be chosen. Both options were found unsuitable; for the first option due to a protein adsorption by the PAC and for the second option due to low and contact time dependent recoveries during extractions of loaded PAC.

A third option involved the assessment of CO$_2$ production as an indirect measure of substrate consumption. Determination of CO$_2$ was accurate and fast, allowing a time evaluation of the status of the experiments. The extent of bioregeneration could be followed without disturbing the process and allowed an accurate observation of the kinetics of growth on sorbed compounds.

A study of the bioregeneration of loaded PAC was performed in batch systems in an experimental set-up comparable to the one used for measuring desorption of PAC-sorbed compounds (de Jonge et al., 1996).
Bioregeneration kinetics

Figure 1 gives typical results of the experiments on the bioregeneration of loaded PAC. Batches of 100-ml mineral salts solutions containing PAC with known 3-CB loadings or containing dissolved 3-CB were inoculated with biomass centrifuged from 10 ml of a continuous culture adapted to growth on 3-CB. Batches of unloaded PAC with adapted biomass were used as blanks.

Several considerations can be made concerning the results presented. Firstly, the CO₂ production curve resulting from growth on CA1-sorbed 3-chlorobenzoic acid has the same shape as the curve of growth on dissolved 3-CB. This means that the desorption of 3-CB from CA1 carbon does not impede growth. Secondly, with growth on SA4-bound 3-CB it can be seen that a slower phase follows the initial phase of fast CO₂ production. Such a phase is absent when growth occurs on dissolved substrate or on CA1-sorbed substrate. Thus with SA4 carbon the bioavailability of sorbed substrate can be a growth-rate limiting factor. A retardation in growth caused by sorbed substrate is in accordance with data reported by Volkering et al. (1992) and Weissenfels et al. (1992). Thirdly, it can be seen that adding a double amount of substrate yields a doubling of the CO₂ produced, which indicates that all the CO₂ comes from the conversion of that substrate. With unloaded powdered activated carbon, SA4 or CA1, no CO₂ production is observed.

The dual-phase character of growth on SA4-sorbed substrate is further confirmed in Fig. 2, where results of growth on SA4-sorbed o-cresol and on dissolved o-cresol are presented. Unloaded SA4 with and without adapted biomass have been used as blanks. In this experiment a smaller quantity of biomass (0.5 ml continuous culture) was used as inoculum. It can be seen, first of all, that growth on SA4-sorbed o-cresol is indeed retarded when compared to growth on dissolved o-cresol, so growth is limited by the availability of SA4-sorbed substrate. Secondly, this effect is more pronounced when the o-cresol has been in contact with SA4 for a longer period. With a contact time of 72 h prior to start of experiment the production of CO₂ is slower and lower than after a contact time of only 2 h, even though the PAC loading is higher with the longer contact time. This indicates that the amount, as well as the kinetics, of availability of the bioregenerable fraction of SA4-sorbed compound decreases with an increasing contact time between PAC and compound. The dual-phase character of growth on SA4-sorbed compound is in close agreement with findings on the kinetics of adsorption and desorption by this type of carbon (de Jonge et al., 1996). Thirdly, growth on dissolved o-cresol showed a second phase of CO₂ production at about 50 h after incubation started. This phenomenon is reproducible and can be hypothetically explained by the consumption of intermediates previously excreted during the phase of high (slightly toxic) o-cresol concentrations, as a reaction to the sudden excess of energy, referred to as overflow metabolism (Neijssel and Tempest, 1975). This is a mechanism to reduce the concentration of o-cresol as fast as possible without overloading the entire metabolic system. In support of this hypothesis, the culture
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Fig. 2. CO₂ production during growth on dissolved and SA4-sorbed o-cresol, with contact times Ct between SA4 and o-cresol of 2 and 72 h prior to the start of the growth experiment. In parentheses the o-cresol concentrations are given in mg/l for dissolved, and in mg/g for SA4-bound o-cresol (SA4 concentration is 1 g/l). Loading batches contained 1 g l⁻¹ SA4 and 300 mg l⁻¹ o-cresol. Blanks are batches with unloaded SA4 with and without biomass. Bioregeneration batches; 100 ml working volume, 215 ml headspace.

A biotic CO₂ production by SA4 carbon

SA4 was found to produce CO₂ independent of biomass (Fig. 2). Study of this phenomenon revealed that this abiotic CO₂ production was influenced by the loading of SA4 with o-cresol. In Fig. 3 the CO₂ production by SA4 after 75 h of incubation is depicted as a function of PAC loading with o-cresol. It is clear that the production of CO₂ by SA4 decreases with an increasing PAC loading with o-cresol, and that this relation is non-linear. Furthermore, it was established that CO₂ production by SA4 occurred only in the presence of EDTA, a chelating agent which is an ingredient of the mineral salts solution used for all experiments involving o-cresol. Microbial degradation of EDTA can not be the cause of this CO₂ production for three reasons. The production of CO₂ also occurred during sterile conditions, biodegradation of EDTA has been looked for thoroughly in these experiments, and was never found, and this abiotic CO₂ production only occurred with SA4 carbon, and not with CA1 carbon. If it were biotic it should also occur in the absence of PAC, or at least with both types of PAC. In the mineral salts solution employed in the experiments with 3-CB, the EDTA concentration was negligible, which explains the absence of CO₂ production by SA4 in these experiments (Fig. 1). It is best to perform all experiments in the same mineral salts solution, in order to

Fig. 3. Influence of PAC loading with o-cresol on the CO₂ production by SA4. CO₂ concentration in headspace was measured after acidification of the batches after 75 h incubation. Contact time prior to incubation was 18 h. All batches 1 g l⁻¹ SA4, 100 ml working volume, 215 ml headspace.
make comparison of results as tenable as possible. However, *Pseudomonas* B13 did not grow on 3-CB as substrate in the medium used for o-cresol: therefore another mineral salts solution, low in EDTA concentration, was adopted for use. When EDTA was added (as a test) to the mineral salts solution used for the experiments with 3-CB, abiotic CO$_2$ production occurred in batches with SA4 carbon, but again not with CA1 carbon.

The nature of the interaction between SA4 carbon and EDTA leading to CO$_2$ production must still be elucidated. To our opinion there are two options possible: (1) EDTA is being (partly) oxidized at the carbon surface of SA4, leading to evolution of CO$_2$. The fact that this would occur with SA4 carbon, but not with CA1 carbon, is in agreement with the finding that oxidative polymerization of o-cresol also seemed to occur with SA4 carbon only (de Jonge et al., 1996). (2) SA4 carbon itself is oxidized in the presence of EDTA, and CA1 is not.

For growth on o-cresol sorbed by SA4, inaccurate substrate consumption data due to the abiotic CO$_2$ production might be obtained. Since the abiotic CO$_2$ production is dependent on the o-cresol PAC loading, blanks with comparable PAC loadings without biomass were used. Furthermore, Fig. 3 shows that the abiotic CO$_2$ production is absent or insignificant when the PAC loading with o-cresol exceeds approximately 100 mg (g PAC)$^{-1}$ (data presented in Fig. 3 are after acidification). So when the PAC loading with o-cresol does not fall below 100 mg (g PAC)$^{-1}$ during bioregeneration, no disturbing influences of the abiotic CO$_2$ production on the measurements are to be expected. Therefore, relatively high PAC loadings were chosen, and the extent of bioregeneration proved not substantial enough to lower the PAC loading so much that abiotic CO$_2$ production might become a problem in the interpretation of the results. For industrial wastewater treatment facilities these high loadings are still realistic, since aromatic compounds can occur in concentrations up to several hundred milligrams per litre (Chao et al., 1986; Janecek and Lamb, 1982).

**Evaluation of CO$_2$ production data**

When the CO$_2$ production curves had reached a stable level, indicating that growth had ceased, the batches were acidified. All CO$_2$ present in the system cumulated in the headspace. Correction with the amount of CO$_2$ present in the corresponding blank after acidification yielded the total amount of CO$_2$ resulting from the consumption of substrate. The pressure in the batches was also measured and compensated for if necessary. To transform these data into amounts of substrate consumed, the CO$_2$ conversion factor (mol CO$_2$ produced per mol compound consumed) was determined by measuring the CO$_2$ production resulting from the consumption of dissolved substrate in the absence of PAC. For o-cresol this specific CO$_2$ yield amounted to 4.01 ± 0.11 mol CO$_2$ per mol o-cresol consumed. For 3-CB the corresponding figure was 3.87 ± 0.28 mol CO$_2$ produced per mol 3-CB consumed. In the case of growth on o-cresol the CO$_2$ yield is in accordance with the biomass growth yield as measured in chemostat experiments (when biodegradation is complete under C-limitation a carbon atom is either converted to CO$_2$ or incorporated into biomass). On a weight to weight basis this yield was 60%. Assuming a C content of biomass on dry weight basis of 50%, this means that 2.7 C-mol is used for biomass growth per mol o-cresol consumed. So 6.7 C atoms of the 7 C atoms in o-cresol are accounted for, a recovery on C-mol basis of 96%. In the case of growth on 3-chlorobenzoic acid the biomass growth yield could not be determined because accurate quantification of the biomass was impossible, due to the fact that the biomass stuck to the walls of the chemostat.

Utilization of the conversion factor assumes a constant yield (C mol biomass produced per C mol substrate consumed, or for that matter, C mol CO$_2$ produced per C mol substrate consumed) with growth on sorbed or dissolved substrate. To validate this assumption, respiration quotients (RQ, in mol CO$_2$ produced per mol O$_2$ consumed) of the various experiments were determined. When mineralization of the substrate is complete, O$_2$ consumed is utilized either for production of CO$_2$ or biomass. Therefore, the RQ can be seen as a control for unchanged yield. From Table 1 it can be concluded that the RQs, and thus the yields, measured by the growth on dissolved substrate are similar to those measured by the growth on sorbed substrate.

**Comparison of SA4 and CA1 with respect to bioregenerability**

Bioregeneration experiments were performed using loaded PAC with various contact times for all four PAC/substrate combinations. The bioregenerable fractions determined are depicted in Figs 4 and 5. From comparison of Figs 4(A) and 5(A) with Figs 4(B) an 5(B), respectively, it is clear that the availability of carbon-sorbed compounds for biodegradation varies considerably with the type of carbon used. The chemically activated, wood-derived CA1 exhibits a larger extent of bioregeneration than the thermally activated, peat-based SA4, for all the contact times tested. It should be noted that the

<table>
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<tr>
<th>Substrate</th>
<th>State of substrate</th>
<th>RQ  (SD)</th>
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<tbody>
<tr>
<td>o-Cresol</td>
<td>Dissolved</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>SA4-sorbed</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>CA1-sorbed</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>3-Chlorobenzoic acid</td>
<td>Dissolved</td>
<td>0.85 ± 0.03</td>
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<tr>
<td></td>
<td>SA4-sorbed</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CA1-sorbed</td>
<td>0.83 ± 0.01</td>
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contact time between the PAC and the compound is of influence for only SA4. Furthermore it can be seen from comparison of Figs 4 and 5 that the extent of bioregeneration is also dependent on the compound adsorbed. With both types of PAC the consumed fraction of sorbed 3-CB is larger than the consumed fraction of sorbed o-cresol.

The bioregeneration mechanism

The data exhibit a considerable variation in the extent of bioregeneration, from 15% of SA4-sorbed o-cresol to over 85% of CA1-sorbed 3-CB. To study the reasons for these differences the bioregenerable fractions of sorbed compound were compared to the desorbable fractions (Figs 4 and 5). The desorbable fractions were determined by exhaustive leaching experiments (de Jonge et al., 1996) under conditions similar as the conditions in the bioregeneration experiment (30°C, pH 7, same mineral salts solutions, same PAC loadings). The leachable fraction represents that amount of the sorbed compound that can be desorbed under abiotic conditions (in the absence of microorganisms). Whereas the bioregenerable fraction is that part of the amount of sorbed compound that can be biodegraded by the microorganisms present.

The bioavailable part of sorbed compound was found to concur with the leachable part for all four PAC/compound combinations. With neither combination did the bioregenerability exceed the leachability. Apparently the bacteria were unable to reach non-desorbable compounds, or to influence the desorbability of the compounds. This indicates that desorption of sorbed compounds will have to occur prior to biodegradation. So bioregeneration is controlled by the desorbability of the compounds loaded onto the PAC. This is further confirmed by the fact that the differences in biodegradation kinetics between both PACs correspond to the differences in desorption kinetics as found earlier (de Jonge et al., 1996).

Although bioregeneration of loaded powdered activated carbon can be expected to play a crucial role in retaining the effectiveness of the PAC–AS system, not many quantitative measurements of bioregeneration have been described in the literature. A comparison of PACs loaded with various compounds with respect to their bioregeneration capacity has to our knowledge not been made previously. Schultz and Keinath (1984) conducted research on the mechanism of the PAC–AS system and found that PAC loaded with phenol could be completely bioregenerated. Speitel and Digiano (1987), in studying the bioregeneration of granular activated carbon (GAC) in filters used for drinking-water treatment, found bioregeneration of loaded GAC varying from 8 to 15% in the case of phenol, and from 5 to 22% in the case of p-nitrophenol. Slow desorption kinetics impeded bioregenera-
rapid decrease of adsorptive capacity during operation in the PAC-AS system. In the longer term, the lesser degree to which the former can be bioregenerated might lead to a more absorptive capacity than CA1 for the model PAC in the PAC-AS system. Although SA4 exhibited 99% bioregeneration, whereas desorbability of compounds loaded with non-desorbable compounds could not be bioregenerated, whereas desorbability of compounds led to bioregeneration. If bioregeneration is controlled through the reversibility of adsorption, this would mean that the degree of regeneration of the loaded PAC could also have been achieved through abiotic desorption, e.g. by leaching of the loaded PAC. Therefore the term “bioregeneration” should be used with caution. Some authors prefer the term to be used only when a direct interaction between microorganisms and the sorbed compound has been demonstrated (Xiaojian et al., 1991). However, most authors define bioregeneration as the process by which the adsorptive capacity of activated carbon is being renewed through the action of microorganisms (Flynn et al., 1976; Chudyk and Snoeyink, 1984; Schultz and Keinath, 1984; Speitel et al., 1989). The last authors reported that GAC loaded with non-desorbable compounds could not be bioregenerated, whereas desorbability of compounds led to bioregeneration.

When several PAC/compound combinations, encompassing a wide range of desorbabilities as in this study, are considered, a more complete picture of the relation between desorption and bioregeneration is obtained. Here a mechanism of bioregeneration where desorption of aromatic compound occurs prior to biodegradation is being very likely. The sorbed compound desorbs when biodegradation results in a decrease of the concentration of dissolved compound in the bulk fluid. Although other mechanisms, such as extracellular enzymes promoting degradation of organic compounds at the carbon surface, have been postulated (Perotti and Rodman, 1974), actual data referring to the mechanism of bioregeneration indicate the validity of the mechanism involving desorption prior to biodegradation (Schulz and Keinath, 1984; Speitel et al., 1989). The last authors reported that GAC loaded with non-desorbable compounds could not be bioregenerated, whereas desorbability of compounds led to bioregeneration.

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**Relevance of bioregenerability to the PAC–AS system**

Major differences are shown to exist in the degree to which the PACs studied can be bioregenerated. The degree of bioregenerability can have important consequences for the functioning and effectivity of PAC in the PAC–AS system. Although SA4 exhibited a higher absorptive capacity than CA1 for the model compounds used, the lesser degree to which the former can be bioregenerated might lead to a more rapid decrease of adsorptive capacity during operation in the PAC–AS system. In the longer term therefore, CA1 might prove to be a better choice, especially during operation under shock-load conditions. Obviously it must be realized that under field conditions the presence of other sorbates, biomass and background organic matter sorption capacities and bioregeneration capacities for individual compounds might not be as high as found in these laboratory experiments. In future, research on the relative importance of bioregenerative capacities compared to adsorptive capacities of the carbons in the PAC–AS process will be investigated in model PAC–AS systems.

**Conclusions**

Based on the data presented the following can be concluded:

1. The maximal extent of bioregeneration is dependent on the type of activated carbon. This fraction is considerably larger with the chemically activated, wood based CA1 than with the thermally activated, peat based SA4.
2. The extent of bioregeneration possible is also dependent on the type of compound sorbed. The phenolic o-cresol was available to a lesser extent than the non-phenolic 3-CB.
3. The availability of a sorbed substrate for biodegradation can decrease with increasing contact time between the substrate and the PAC.
4. Bioregeneration is controlled by the reversibility of adsorption, which means that the mechanism of bioregeneration for the PAC/compound combinations tested is desorption prior to biodegradation.
5. Desorption kinetics can be the rate-limiting step in the microbial degradation of a sorbed compound.
6. Bacteria were unable to influence the desorbability of the compounds tested.

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**References**


Ehrhardt H. M. and Rehm H. J. (1985) Phenol degradation of GAC loaded with 2,4-dichlorophenol (Speitel et al., 1989). Others found more substantial percentages of bioregeneration of GAC loaded with phenol; Ehrhardt and Rehm (1985) found 90% bioregeneration, and Hutchinson and Robinson (1990) 82% bioregeneration. With 3-CB loaded carbon Jaar and Wilderer (1992) and Jaar et al. (1989) found 100% bioregeneration after 67 days.

These reports on bioregeneration were concerned with one compound and one species of carbon. When several PAC/compound combinations, encompassing a wide range of desorbabilities as in this study, are considered, a more complete picture of the relation between desorption and bioregeneration is obtained.

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