BIOREGENERATION OF ACTIVATED CARBON IN THE TREATMENT OF PHENOLIC COMPOUNDS

by

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ABSTRACT

This study aimed to clarify the effect of adsorbability, desorbability, biodegradability and activated carbon type on the extent of bioregeneration in the treatment of phenolic compounds in activated sludge systems combined with activated carbon. Bioregenerabilities of activated carbons preloaded with phenol, 2-chlorophenol and 2-nitrophenol were studied. For this purpose, four different activated carbon types; thermally activated and chemically activated powdered carbons (PAC), and their granular countertypes (GAC) with similar physical characteristics were used. Thermally activated carbons were better adsorbers for phenolic compounds than chemically activated ones. However, apparently higher adsorption irreversibility was calculated in the case of thermally activated carbons. The results suggested that, rather than the physical form, carbon activation type and chemical surface characteristics played a more important role on adsorbability of phenolic compounds and its reversibility. Also, adsorption and its reversibility were highly dependent on the type of the target compound. Bioregeneration of chemically activated carbons was also higher than thermally activated ones. This showed that bioregeneration was controlled by the reversibility of adsorption. The results suggested that carbon activation type was of crucial importance for bioregeneration. PAC and GAC countertypes showed comparable bioregeneration efficiencies indicating that carbon size was not an effective factor. Oxidative polymerization of phenol and 2-chlorophenol was a plausible explanation for lesser bioregeneration of thermally activated carbons. However, bioregeneration efficiencies of thermally activated carbons were much higher than their total desorbabilities. This indicated the presence of exoenzymatic bioregeneration. Bi-solute experiments showed that competition for adsorption sites greatly determined the extent of adsorption reversibility and bioregeneration. Cometabolic biodegradation of 2-chlorophenol and 2-nitrophenol in the presence of phenol resulted in efficient bioregenerability of activated carbons, when they were loaded with non-growth substances together with a growth substrate.

ÖZET

Bu çalışmanın amacı, aktif çamur ve aktifkarbonun birarada bulunması halinde, fenolik maddelerin gideriminde, adsorplanabilirlik, desorplanabilirlik, biyolojik olarak ayrıştırılabilirliğin ve aktif karbon tipinin biyorejenerasyon mertebesine olan etkisini ortaya koymaktır. Çalışmada, fenol, 2-klorofenol ve 2-nitrofenol ile önceden yüklenmiş aktif karbonların biyorejenere edilebilirliği araştırılmıştır. Bu amaçla termal ve kimyasal olarak aktive edilmiş toz aktif karbonlar ve bunların benzer fiziksel özelliklere sahip granüler eşleri kullanılmıştır. Termal olarak aktive edilmiş karbonlar fenolik maddeleri daha iyi adsorplamışlardır. Ancak termal olarak aktive edilmiş karbonlarda adsorpsiyonun tersinirliğinin daha düşük olduğu bulunmuştur. Sonuçlar göstermiştir ki, aktif karbonun fiziki formundan ziyade, aktivasyon yöntemi ve kimyasal yüzey özellikleri, fenolik bileşiklerin adsorpsiyonunu ve bunun tersinirliğini daha fazla etkilemektedir. Aynı zamanda, adsorpsiyonun ve tersinirliğinin hedef bileşiğe göre de değiştiği bulunmuştur. Kimyasal olarak aktive edilmiş karbonlar, yüksek adsorpsiyon tersinirliklerine bağlı olarak daha fazla biyorejenerasyona uğramışlardır. Bu da biyorejenerasyonun adsorpsiyonun tersinirliğine bağlı olduğunu göstermiştir. Sonuçlar, karbon aktivasyon yönteminin biyorejenerasyon açısından çok önemli olduğunu göstermiştir. Toz ve granüler aktif karbon eşlerinde biyorejenerasyon verimlerinin birbiriyle karşılaştırılabilir olması, partikül büyüklüğünün önemli bir etken olmadığını göstermiştir. Fenol ve 2-klorofenolün oksidatif polimerizasyonu termal olarak aktive edilmiş karbonların daha az biyorejenere olmalarına neden olmaktadır. Ancak, termal olarak aktive edilmiş karbonların biyorejenerasyon verimleri toplam desorplanabilirliklerinden fazla bulunmuştur. Bu da hücre dışındaki enzimlere bağlı bir biyorejenerasyonun varlığına işaret etmektedir. İki bileşikle yapılan deneyler göstermiştir ki, bileşiklerin adsorpsiyon için rekabet etmesi, adsorpsiyonun tersinirliğini ve biyorejenerasyonun mertebesini belirlemektedir. Fenolün varlığında, 2-klorofenol ve 2-nitrofenol kometabolik olarak ayrıştırılabilmiştir. Bu da biyolojik ayrıştırılabilirliği zor olan maddelerle yüklenmiş aktif karbonların biyolojik olarak giderilebilen maddelerin varlığında biyorejenerasyonunu mümkün kılmıştır.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii
ABSTRACT iv
ÖZET v
TABLE OF CONTENTS vi
LIST OF TABLES xi
LIST OF FIGURES xiii
LIST OF SYMBOLS/ABBREVIATIONS xix
1. INTRODUCTION 1
1.1. Background of the Problem 1
1.2. Aim and Scope of the Study 3
2. THEORETICAL BACKGROUND 4
2.1. Activated Carbon Adsorption 4
2.1.1. Definition 4
2.1.2. The Relationship Between Surface Tension and Adsorption 4
2.1.3. Types of Adsorption 5
2.1.4. Factors Influencing Adsorption 6
2.1.4.1. Surface Area of Adsorbent 6
2.1.4.2. Nature of Adsorbate 6
2.1.4.3. pH 7
2.1.4.4. Temperature 7
2.1.5. Adsorption Equilibrium and Isotherms 7
2.1.6. Activated Carbon 8
2.1.6.1. Preparation of Activated Carbons 8
2.1.6.2. Activated Carbon Characteristics 9
2.1.6.3. Powdered Activated Carbon 11
2.1.6.4. Granular Activated Carbon 11
2.2. Biological Treatment of Phenolic Compounds 12
2.2.1. Biodegradation of Phenolic Compounds 12
2.2.2. Cometabolic Degradation of Inhibitory Phenolic Compounds 14
2.3. Bioregeneration of Activated Carbon 18

2.3.1. Enhancement of Biological Treatment by Activated Carbon Adsorption 18
2.3.2. Bioregeneration Theories 20
2.3.3. Dependency of Bioregeneration on Reversibility of Adsorption 25
2.3.4. Factors Affecting Bioregeneration 28
2.3.4.1. Biodegradability 29
2.3.4.2. Substrate Type 29
2.3.4.3. Carbon Particle Size 30
2.3.4.4. Carbon Porosity 30
2.3.4.5. Carbon Activation Type 31
2.3.4.6. Physical Surface Properties of Carbon 31
2.3.4.7. Desorption Kinetics 32
2.3.4.8. Substrate- Carbon Contact Time 32
2.3.4.9. Concentration Gradient and Carbon Saturation 33
2.3.4.10. Biomass Concentration 35
2.3.4.11. Dissolved Oxygen Concentration 35
2.3.4.12. Microorganism Type 35
2.3.4.13. Substrate and Biomass Associated Products of Biodegradation 36
2.3.5. Determination of Bioregeneration 37
2.3.5.1. Investigation of the Extent of Reversible Adsorption and Bioregeneration 37
2.3.5.2. Investigation by Scanning Electron Microscopy 38
2.3.5.3. Investigation of Bioregeneration in Multiple Substrate Systems 39
2.3.5.4. Bioregeneration of Adsorbents other than Activated Carbon 40
2.3.6. Quantification of Bioregeneration 41
2.3.6.1. Use of Freundlich Adsorption Analogy 41
2.3.6.2. Direct Measurement by using Adsorption Capacities 43
2.3.6.3. Direct Measurement by Solvent Extraction 43
2.3.6.4. Quantification during Simultaneous Adsorption 44
2.3.6.5. Use of Measurement of Biodegradation Products 44
2.3.6.6. Use of Respirometry in Aerobic Systems 45

2.3.6.7. The Kinetics of Bioregeneration 46
2.3.7. Mathematical Modelling Approaches 46
2.3.8. Overall Discussions on Literature Work 49
3. MATERIALS AND METHODS 51
3.1. Activated Carbon Characteristics 51
3.2. Characteristics of the Target Phenolic Compounds 52
3.3. Experiments with Phenol 54
3.3.1. Adsorption Experiments with Phenol 54
3.3.2. Desorption Experiments with Phenol 54
3.3.3. Biodegradation and Bioregeneration Experiments with Phenol 55
3.4. Experiments with 2-Chlorophenol 59
3.4.1. Adsorption Experiments with 2-Chlorophenol 59
3.4.2. Desorption Experiments with 2-Chlorophenol 59
3.4.3. Biodegradation and Bioregeneration Experiments with 2-Chlorophenol 59
3.5. Experiments with a Mixture of Phenol and 2-Chlorophenol 60
3.5.1. Adsorption Experiments with a Mixture of Phenol and 2-Chlorophenol 60
3.5.2. Desorption Experiments with a Mixture of Phenol and 2-Chlorophenol 61
3.5.3. Biodegradation and Bioregeneration Experiments with a Mixture of Phenol and 2-Chlorophenol 61
3.6. Experiments with 2- Nitrophenol 64
3.6.1. Adsorption Experiments with 2-Nitrophenol 64
3.6.2. Desorption Experiments with 2-Nitrophenol 64
3.6.3. Biodegradation and Bioregeneration Experiments with a Mixture of Phenol and 2-Nitrophenol 64
3.7. Analytical Methods 66
4. RESULTS AND DISCUSSIONS 70
4.1. Experiments with Phenol as a Model Compound 70
4.1.1. Adsorption Studies with Phenol 70
4.1.2. Desorption Studies with Phenol 75
4.1.3. Biodegradation and Bioregeneration Studies with Phenol 81

4.1.3.1. Phenol and COD removals 81
4.1.3.2. Investigation of Bioregeneration by Oxygen Uptake Rates 82
4.1.3.3. Quantification of Bioregeneration 91
4.1.3.4. Bioregeneration due to Concentration Gradient 93
4.1.3.5. Bioregeneration due to Exoenzymatic Reactions 94
4.1.3.6. Oxidative Coupling of Phenol 97
4.1.3.7. ESEM Analysis of Carbon Surfaces 98
4.2. Experiments with 2-Chlorophenol as a Model Compound 101
4.2.1. Adsorption Studies with 2-Chlorophenol 101
4.2.2. Desorption Studies with 2-Chlorophenol 105
4.2.3. Biodegradation and Bioregeneration Studies with 2-Chlorophenol 108
4.2.3.1. The use of Non-acclimated Biomass 108
4.2.3.2. The use of Acclimated Biomass 110
4.3. Experiments with a Bi-solute Mixture of Phenol and 2-Chlorophenol 113
4.3.1. Competitive Adsorption Studies 113
4.3.2. Description of Competitive Adsorption using Models 118
4.3.3. Desorption Studies 122
4.3.4. Competitive Biodegradation and Bioregeneration Studies 127
4.3.4.1. Bi-Solute Biodegradation of Phenol and 2-Chlorophenol 127
4.3.4.2. Removal of Phenol and 2- Chlorophenol in Bioregeneration Reactors 134
4.3.4.3. Investigation of Bioregeneration by OURs and Chloride Formation 138
4.3.4.4. Quantification of Bioregeneration 140
4.3.4.5. Investigation by Environmental Scanning Electron Microscopy (ESEM) 143
4.4. Experiments with 2-Nitrophenol as a Model Compound 147
4.4.1. Adsorption Studies with 2-Nitrophenol 147
4.4.2. Desorption Studies with 2-Nitrophenol 149
4.4.3. Biodegradation and Bioregeneration Experiments with 2-Nitrophenol 151
4.4.3.1. Biodegradation of 2-Nitrophenol 151

4.4.3.2. Removal of Phenol and 2-Nitrophenol in Bioregeneration Reactors. 156
4.4.3.3. Quantification of Bioregeneration 158
5. CONCLUSIONS AND RECOMMENDATIONS 162
REFERENCES 168
APPENDIX A: CALIBRATION CURVES AND CHROMATOGRAMS 185
APPENDIX B: ESEM IMAGES 189

LIST OF TABLES

Table 2.1.	Examples of bioregeneration efficiencies and process conditions reported in literature	42
Table 3.1.	Characteristics of activated carbons used in the experiments	51
Table 3.2.	Properties of the model phenolic compounds used in the experiments	52
Table 3.3.	Main characteristics of biodegradation and bioregeneration experiments with phenol as the target compound	58
Table 3.4.	Main characteristics of biodegradation and bioregeneration experiments with 2-chlorophenol (2-CP) as the target compound	60
Table 3.5.	Main characteristics of biodegradation and bioregeneration experiments with a mixture of phenol and 2-chlorophenol	63
Table 3.6.	Main characteristics of biodegradation and bioregeneration experiments with a mixture of phenol and 2-nitrophenol	65
Table 4.1.	Freundlich isotherm constants for phenol adsorption	70
Table 4.2.	Langmuir isotherm constants for phenol adsorption	73
Table 4.3.	Conditions of preliminary adsorption preceding desorption experiments and Freundlich desorption isotherm constants for phenol	77
Table 4.4.	Adsorption and desorption efficiencies of phenol for each activated carbon type	78
Table 4.5.	Bioregeneration rates and efficiencies for activated carbons loaded with phenol	92
Table 4.6.	Freundlich isotherm constants for 2-chlorophenol adsorption	101
Table 4.7.	Langmuir isotherm constants for 2-chlorophenol adsorption	104
Table 4.8.	Freundlich isotherm constants for 2-chlorophenol desorption	106
Table 4.9.	Adsorption and desorption efficiencies of 2-chlorophenol for each activated carbon type	107
Table 4.10.	Competitive adsorption Freundlich isotherm constants for 2-chlorophenol in the presence of phenol	115
Table 4.11.	Competitive adsorption Langmuir isotherm constants for 2-chlorophenol in the presence of phenol	117

Table 4.12.	ISIAS model competition factors for phenol and 2-CP	121
Table 4.13.	Freundlich desorption isotherm constants for competitively adsorbed phenol and 2-CP	123
Table 4.14.	Competitive adsorption and desorption efficiencies for bi-solute mixtures of phenol and 2-CP	125
Table 4.15.	Bioregeneration rates and efficiencies for activated carbons competitively loaded with phenol and 2-chlorophenol	140
Table 4.16.	Freundlich isotherm constants for 2-nitrophenol adsorption	148
Table 4.17.	Langmuir isotherm constants for 2-nitrophenol adsorption	149
Table 4.18.	Freundlich isotherm constants for 2-nitrophenol desorption	150
Table 4.19.	Bioregeneration rates and efficiencies for activated carbons competitively loaded with phenol and 2-nitrophenol	159

LIST OF FIGURES

Figure 2.1.	Graphite structure (a,b) and turbostratic structure (c) of activated carbon. Conceptual illustration of granular activated carbon (d) (Suzuki, 1990)	10
Figure 2.2.	Typical oxygenase enzyme reactions for growth-substrate and cometabolic (non-growth) substrate (Chang and Alvarez-Cohen, 1995).	17
Figure 3.1	Molecular structures of phenolic compounds used in experiments	53
Figure 3.2.	Simultaneously operated batch activated sludge reactors; biodegradation reactor without activated carbon and bioregeneration reactor with loaded activated carbon	56
Figure 3.3.	Environmental scanning electron microscopy of microorganisms and flocs in non-acclimated activated sludge taken from mother reactor.	57
Figure 3.4.	Environmental scanning electron microscopy of microorganisms in activated sludge acclimated with phenol and 2- CP	62
Figure 3.5.	Environmental scanning electron microscopy of microorganisms in activated sludge acclimated with phenol and 2- NP	66
Figure 4.1	Phenol adsorption isotherms for powdered activated carbons SA4 (thermally activated) and CA1 (chemically activated) and granular carbons PKDA (thermally activated) and CAgran (chemically activated) with their Freundlich and Langmuir fits	71
Figure 4.2.	Effect of activated carbon dose on equilibrium phenol concentrations with an initial phenol concentration of 200 mg/L	72
Figure 4.3.	Phenol desorption isotherms for powdered activated carbons SA4 (thermally activated) and CA1 (chemically activated) at doses of 2000 mg/L and 4000 mg/L	76
Figure 4.4.	Phenol desorption isotherms for granular carbons PKDA (thermally activated) and CAgran (chemically activated) at doses of 2000 mg/L and 4000 mg/L	77
Figure 4.5.	Desorption of phenol from the four loaded activated carbons with respect to time	79

Figure 4.6. Cumulative desorption of phenol from loaded activated carbons at the end of each successive desorption step 80

Figure 4.7. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 1000 mg/L SA4 (powdered , thermally activated) in RUN 1 83

Figure 4.8. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L SA4 (powdered , thermally activated) in RUN 2 84

Figure 4.9. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L CA1 (powdered , chemically activated) in RUN 3 85

Figure 4.10. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L PKDA (granular, thermally activated) in RUN 4 86

Figure 4.11. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L CAgran (granular, chemically activated) in RUN 5 87

Figure 4.12. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L SA4 (powdered, thermally activated) and CA1 (powdered, chemically activated) in RUN 6 88

Figure 4.13. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L PKDA (granular, thermally activated) and CAgran (granular, chemically activated) in RUN 7 89

Figure 4.14. Profiles of phenol loadings on activated carbons SA4 (powdered, thermally activated), CA1 (powdered, chemically activated), PKDA (granular, thermally activated) and CAgran (granular, chemically activated) a) in bioregeneration reactors in RUNS 2-7 and b) during desorption equilibrium 96

Figure 4.15. Scanning Electron Micrographs of microorganisms on activated carbon a) filamentous bacteria b) spiral-shaped bacteria c) a protozoan d) a spherical protozoan attached to carbon surface e)group of cocci-shaped bacteria clustered in the carbon cavities f) a stalked protozoan attached to carbon cavities 99

Figure 4.16. Scanning Electron Micrographs of a) virgin dry SA4 b) virgin dry CA1 c) slime matrix covering parts of carbon surface forming a bright white gelatinous structure d) Slime matrix covering microorganisms on activated carbon 100

Figure 4.17. 2-Chlorophenol profiles during adsorption at an initial 2-CP concentration of 200 mg/L and 1 g/L of activated carbons SA4, CA1, PKDA and CAgran 102

Figure 4.18. 2-Chlorophenol adsorption isotherms for each activated carbon type SA4 (powdered, thermally activated), CA1 (powdered, chemically activated), PKDA (granular, thermally activated) and CAgran (granular, chemically activated) 103

Figure 4.19. Effect of activated carbon dose on equilibrium 2-chlorophenol concentrations with an initial 2-chlorophenol concentration of 200 mg/L for each activated carbon type 103

Figure 4.20. 2-Chlorophenol profiles during desorption at a dose of 2 g/L of activated carbons SA4, CA1, PKDA and CAgran 105

Figure 4.21. 2-Chlorophenol desorption isotherms for activated carbon types SA4, CA1, PKDA and CAgran 106

Figure 4.22. Cumulative desorption of 2-chlorophenol from loaded activated carbons at the end of each successive desorption step (SA4: powdered, thermally activated, CA1: powdered, chemically activated, PKDA: granular, thermally activated and CAgran: granular, chemically activated) 108

Figure 4.23. a) 2-Chlorophenol and COD profiles and b) OUR profiles in biodegradation and bioregeneration reactors in RUN 8 with 2-chlorophenol when non-acclimated biomass and SA4 carbon were used 111

Figure 4.24. 2-Chlorophenol and COD profiles in biodegradation and bioregeneration reactors in RUN 9 with 2-chlorophenol when acclimated biomass and SA4 carbon were used 112

Figure 4.25. Competitive bi-solute and single-solute adsorption isotherms for phenol and 2-CP for a) SA4, b)PKDA, c) CA1, d)CAgran. (open data points refer to single-solute adsorption, closed data points refer to bi-solute adsorption) 114

Figure 4.26. Effect of activated carbon dose on equilibrium phenol and 2-CP concentrations in competitive bi-solute and single-solute adsorption studies with initial concentrations of 200 mg/L for a) SA4, b) PKDA, c) CA1, d) CAgran. (open data points refer to single-solute adsorption, closed data points refer to bi-solute adsorption). 116

Figure 4.27. Competitive bi-solute isotherms for phenol and 2-CP with their Simplified Ideal Adsorbed Solution (SIAS) and Improved SIAS (ISIAS) model fits for a) SA4, b) PKDA, c) CA1, d) CAgran. (full lines refer to SIAS model fits, dashed lines refer to ISIAS model fits). 120

Figure 4.28. Competitive desorption isotherms of each activated carbon type for a) phenol and b) 2-CP 124

Figure 4.29. Cumulative desorption of a) phenol and b) 2-CP from competitive loaded activated carbons at the end of each successive desorption step 127

Figure 4.30. a) Phenol , 2-CP and COD profiles b) OUR and chloride profiles in the biodegradation and bioregeneration reactor with 2000 mg/L SA4 (powdered, thermally activated) in RUN 10 129

Figure 4.31. a) Phenol , 2-CP and COD profiles b) OUR and chloride profiles in the biodegradation and bioregeneration reactor with 2000 mg/L CA1 (powdered, chemically activated) in RUN 11 130

Figure 4.32. a) Phenol , 2-CP and COD profiles b) OUR and chloride profiles in the biodegradation and bioregeneration reactor with 2000 mg/L PKDA (granular, thermally activated) in RUN 12 131

Figure 4.33. a) Phenol , 2-CP and COD profiles b) OUR and chloride profiles in the biodegradation and bioregeneration reactor with 2000 mg/L CAgran (granular, chemically activated) in RUN 13 132

Figure 4.34. a) Phenol , 2-CP and COD profiles b) OUR and chloride profiles in the and bioregeneration reactors with SA4 (powdered, thermally activated) and CA1 (powdered, chemically activated) in RUN 14 136
Figure 4.35. a) Phenol , 2-CP and COD profiles b) OUR and chloride profiles in the and bioregeneration reactors with PKDA (granular, thermally activated) and CAgran (granular, chemically activated) in RUN 15 137
Figure 4.36. Scanning Electron Micrographs of microorganisms on SA4in RUN 14 144
Figure 4.37. Scanning Electron Micrographs of microorganisms on CA1 in RUN 14 144
Figure 4.38. Scanning Electron Micrographs of microorganisms on PKDA in RUN 15 145
Figure 4.39. Scanning Electron Micrographs of microorganisms on CAgran in RUN 15 145
Figure 4.40. 2-Nitrophenol profiles during adsorption onto 1 g/L of activated carbons SA4 and CA1 147
Figure 4.41. 2-Nitrophenol adsorption isotherms for activated carbon types SA4 and CA1 148
Figure 4.42. 2-Nitrophenol desorption isotherms for activated carbon types SA4 and CA1 150
Figure 4.43. a) Phenol and 2-NP profiles b) COD and OUR profiles in the biodegradation reactors receiving acclimated (Acc. AS) and non acclimated (Non-Acc.AS) activated sludges in RUN 16 152
Figure 4.44. a) Phenol and 2-NP profiles b) COD and OUR profiles in the biodegradation reactor and bioregeneration reactor with 2000 mg /L SA4 (powdered, thermally activated) in RUN 17 153
Figure 4.45. a) Phenol and 2-NP profiles b) COD and OUR profiles in biodegradation reactor (receives 2-NP as the only carbon source) and bioregeneration reactor with 2000 mg/L CA1 (powdered, chemically activated) in RUN 18 154
Figure 4.46. a) Phenol and 2-NP profiles b) COD and OUR profiles in the bioregeneration reactors with SA4 (powdered, thermally activated) and CA1 (powdered, chemically activated) in RUN 19 157
Figure A.1. Calibration curve for high range chemical oxygen demand (COD) analysis 185

Figure A.2. Calibration curve for low range chemical oxygen demand (COD) analysis 185
Figure A.3. Calibration curve for phenol analysis with the 4-aminoantipyrine method within the concentration range of 0.1-1 mg/L 186
Figure A.4. Calibration curve for phenol analysis with the 4-aminoantipyrine method within the concentration range of 1-5 mg/L 186
Figure A.5. Calibration curves for gas chromatographic analysis 187
Figure A.6. Example of a gas chromatogram for a sample containing 200 mg/L of each compound; phenol, 2-chlorophenol (2-CP) and 2-nitrophenol (2-NP) 187
Figure A.7. Gas chromatogram of mixed phenols standard (each 20 mg/L in methanol) 188
Figure B.1. Environmental scanning electron microscopy of non-acclimated activated sludge taken from the mother reactor 189
Figure B.2. Environmental scanning electron microscopy of activated sludge acclimated to phenol and 2-chlorophenol 190
Figure B.3. Environmental scanning electron microscopy of activated sludge acclimated to phenol and 2-nitrophenol 191
Figure B.4. Environmental scanning electron micrographs of microorganisms on SA4 carbon at t=3 days in RUN 14 192
Figure B.5. Environmental scanning electron micrographs of microorganisms on CA1 carbon at t=3 days in RUN 14 193
Figure B.6. Environmental scanning electron micrographs of microorganisms on PKDA carbon at t=3 days in RUN 15 194
Figure B.7. Environmental scanning electron micrographs of microorganisms on CAgran at t=3 days in RUN 15 195
Figure B.8. Environmental scanning electron micrographs of microorganisms on CAgran at t=8 days in RUN 15 195

LIST OF SYMBOLS/ABBREVIATIONS

a_1, a_2 : competition factors

b : Langmuir constant related to the energy of adsorption, L/mg

BAC : Biological Activated Carbon

BASM : Biodegradation/Adsorption Screening Model

BOD : Biochemical Oxygen Demand, mg/L

C : the measured concentration in solution at equilibrium, mg/L

C_o : the initial adsorbate concentration, mg/L

C_1 : the initial concentration in the bulk, mg/L

C_e : the equilibrium concentration in the bulk, mg/L

C_e' : the adsorbate concentration in the bulk liquid during bioregeneration, mg/L

C_{2e} : the equilibrium concentration after post-bioregeneration adsorption, mg/L

COD : Chemical Oxygen Demand, mg/L

2-CP : 2-chlorophenol

4-CP : 4-chlorophenol

CSTR : Completely Stirred Tank Reactor

2,4-DCP : 2,4-dichlorophenol

EBCT : Empty Bed Contact Time

ESEM : Environmental Scanning Electron Microscopy

$\Delta G°_{ads}$: Gibbs free energy of adsorption

GAC : Granular Activated Carbon

HMW : High Molecular Weight

IAS : Ideal Adsorbed Solution

IAST : Ideal Adsorbed Solution Theory

ISIAS : Improved Simplified Ideal Adsorbed Solution

K : Freundlich constant, $[mg/g)\ (L/mg)^{1/n}]$

K_i : Single-solute empirical Freundlich constant for solute i, $[mg/g)\ (L/mg)^{1/n}]$

K’ : average value of K_i, $[mg/g)\ (L/mg)^{1/n}]$

k : first-order bioregeneration rate constant, h^{-1}

LMW : Low Molecular Weight

M : mass of activated carbon used in each desorption experiment, g.

MDBA : multiple component biofilm diffusion biodegradation and adsorption model

MLSS : Mixed Liquor Suspended Solids, mg/L

MLVSS : Mixed Liquor Volatile Suspended Solids, mg/L

OUR : Oxygen Uptake Rate, mg O_2/L.h

NAD(P)H : nicotineamide adenine dinucleotide (phosphate) reduced

2-NP : 2-nitrophenol

1/n : Freundlich constant

n_i : single-solute empirical Freundlich constant for solute i

n' : average value of n_i

PAC : Powdered Activated Carbon

PACT : Powdered Activated Carbon Treatment

pK_a : dissociation constant

Q_1 : the initial amount of adsorbate adsorbed on activated carbon, mg

Q_2 : the amount of adsorbate remained after contact with biomass, mg

Q° : the amount solute adsorbed in forming a complete monolayer, mg/g

Q′ : the amount of adsorbed substrate left on activated carbon, mg

ΔQ_2 : the additional amount of substrate adsorbed after bioregeneration, mg

q : adsorption capacity of the activated carbon, mg/g

q_i : hypothetical activated carbon loading in desorption steps, mg/g

R_L : dimensionless separation factor

RPM : Revolution Per Minute

RSS : residual sum of squares

S_e : the equilibrium adsorbate concentration, mg/L

S_0/X_0 : initial substrate/initial biomass ratio

SBR : Sequencing Batch Reactor

SCOD : soluble COD (COD of filtrated sample), mg/L

SEM : Scanning Electron Microscopy

SIAS : Simplified Ideal Adsorbed Solution

SOC : Synthetic Organic Chemicals

SMP : Soluble Microbial Products

SRT : Sludge Residence Time, d

TCE : trichloroethylene

V : total volume of sample, L

W : weight of activated carbon, g

W (%) : degree of hysteresis

X : average biomass concentration, mg MLSS/L

X_a : initial adsorbate loading on the carbon, mg

X_d : mass of phenol desorbed after each i^{th} desorption step, mg

α : equilibrium adsorption capacitiy of fresh activated carbon, mg/g

α : equilibrium adsorption capacitiy of fresh activated carbon, mg/g

$\alpha_{reg.}$: equilibrium adsorption capacitiy of regenerated activated carbon, mg/g

δ : sorbate solubility, %

Φ : pore diameter, nm

1. INTRODUCTION

1.1. Background of the Problem

Aromatic compounds are widespread pollutants present in many industrial effluents due to their extensive use. They can occur in concentrations up to several hundred miligrams per liter (Jonge et al., 1996a). Phenolic compounds commonly appear in the environment as a result of both humification processes and anthropogenic activities. Phenolic hydrocarbons are widely used in pharmaceutical, petrochemical, pulping industries and other chemical manufacturing processes (Chern and Chien, 2002). Aqueous waste streams from petroleum refineries and coal processing industries often contain phenolic compounds at a level of 500-10000 mg/L (Ha and Vinitnantharat, 2000). The urine of some animals and decay of vegetation also release phenol to water bodies. Phenolic compounds can cause objectionable taste and odor problems in drinking water (Vidic et al., 1993).

Chlorophenols are an important class of aromatic pollutants in industrial wastewaters because of their wide use in the production of preservers, pesticides and biocides (Quan et al., 2005). Chlorinated phenols can exist up to 100-200 mg/L in contaminated environments (Sahinkaya and Dilek, 2005). Nitrophenols also exist in wastewaters of pharmaceutical, petrochemical and other manufacturing processes (Chern and Chien, 2002). Due to their strong toxicity, persistence in the environment and suspected carcinogenicity (Quan et al., 2005), wastewaters containing phenolic compounds should be treated before discharge into water bodies.

Phenolic compounds are major target compounds in wastewater treatment systems, since they are usually poorly degraded in conventional wastewater treatment systems. Treatment methods for removing phenolic compounds include both aerobic and anaerobic biodegradation, chemical oxidation, adsorption by ion exchange resin and activated carbon adsorption (Vidic et al., 1993). Phenolic compounds can exert adverse effects on various biological treatment processes due to strong toxicity to indigenous microorganisms and the poor number of specific microorganisms capable of degrading them (Quan et al., 2005).

However, they can be very effectively removed by activated carbon adsorption (Kolb and Wilderer, 1997). Therefore combination of biological treatment with activated carbon adsorption is a promising method for the removal of phenolic compounds. Particularly, bioregenerability of activated carbon facilitates their removal in these systems without increasing costs (Jonge et al., 1996a).

Activated carbon in conjunction with biological treatment has been widely used for treatment of wastes containing non-biodegradable and toxic organics over the past 30 years. Combination of activated carbon with biological treatment can be achieved either by addition of powdered activated carbon (PAC) to activated sludge or by granular activated carbon (GAC) filters covered by biofilm. Activated carbon is applied in the treatment of highly polluted wastewaters contaminated with phenols, aromatics, volatile acids, aliphatics besides general organic matter reduction.

The effectiveness of activated carbon in removing contaminants from solution is dependent on the type of activated carbon used and the characteristics of the target solutes. Several types have been developed for specific ranges of applications, from materials such as wood, coconut husks, lignite and bituminous coal. The process of activation at high temperatures creates a material comprising micropores and mesopores in varying ratios depending on the parent material and the method of activation (thermal or chemical). This creates a large internal surface, which has a high degree of surface reactivity (Garner et al., 2001).

In all activated carbon applications, the available adsorption sites become exhausted with adsorbed pollutants and the activated carbon loses its adsorptive capacity. The activated carbon when used alone (GAC filters) causes a large capital investment and operating cost, mainly due to the need for regeneration. One method for the regeneration of spent activated carbon is bioregeneration of activated carbon. Bioregeneration can be defined as the renewal of the adsorptive capacity of activated carbon by biodegradation of previously adsorbed organic matter for further adsorption (Jonge et al., 1996a). Thus, the bioregeneration process increases the service period of activated carbon. Bioregeneration occurring in PAC added activated sludge and BAC (Biological activated carbon) systems may decrease the costs related with regeneration and replacement of activated carbon by

extending the adsorption capacity of PAC or GAC and increasing the service period**.** Bioregeneration process requires desorbability of the adsorbed compounds and subsequent biodegradability of these compounds (Jonge et al., 1996a).

There is a lack of knowledge in literature about the effect of both carbon activation type and physical form (PAC or GAC) on reversibility of adsorption and bioregeneration. Also, the type of the target compound and the presence of multiple substrates were not investigated extensively within the subject of bioregeneration. An extensive literature review (Section 2.3) on bioregeneration showed that there was no study in literature considering all of these factors and the interrelationship between them.

1.2. Aim and Scope of the Study

This study aims to clarify the effect of substrate type, presence of multiple substrates, activated carbon type and physical form of carbon on the extent of adsorbability and desorbability of phenolic compounds and bioregeneration of activated carbon. For this purpose, two different PACs; thermally activated Norit SA4 and chemically activated Norit CA1, and their granular countertypes with similar physical characteristics, thermally activated Norit PKDA and chemically activated Norit CAgran were used for determination of the extent of adsorbability, reversibility of adsorption and bioregeneration. As model compounds phenol, 2-chlorophenol and 2-nitrophenol were used. Phenol was selected over others as a model compound, because it is both adsorbable and biodegradable which enabled to study the interaction between the activated carbon and biomass. Because of their very low biodegradability compared to phenol, 2-chlorophenol and 2-nitrophenol were selected as other target compounds in order to investigate the effect of substrate type on the extent of bioregeneration. The aim for 2-nitrophenol selection over other phenolic compounds was to investigate the effect of the presence or absence of oxidative coupling reactions on reversibility of adsorption and bioregeneration. Nitrophenols are the only phenolic compounds, which do not undergo oxidative coupling reactions. Bi-solute adsorption and desorption experiments with phenol and 2-chlorophenol enabled the investigation of competitive adsorption and its reversibility. Bi-solute biodegradation and bioregeneration experiments with pairs of phenol-2-chlorophenol and phenol-2-nitrophenol enabled the investigation of competitive biodegradation and bioregeneration.

2. THEORETICAL BACKGROUND

2.1. Activated Carbon Adsorption

2.1.1. Definition

Adsorption is recognized as a significant phenomenon in most natural physical, biological and chemical processes. Sorption on solids has become a widely used operation for purification of wastewaters. Activated carbon is the most widely used material in wastewater treatment technologies.

Adsorption is the interface accumulation or concentration of substances at a surface or interface. Adsorption can occur between any two phases, such as, liquid-liquid, gas-liquid, gas-solid, or liquid-solid interfaces. The material being concentrated or adsorbed is termed the adsorbate, and the adsorbing phase is termed the adsorbent.

2.1.2. The Relationship Between Surface Tension and Adsorption

Adsorption is a surface phenomenon in which matter is extracted from one phase and concentrated at the surface of a second phase. Such surface reactions must occur as a result of the active forces within the phase or surface boundaries. These forces result in characteristic boundary energies.

A pure liquid drop placed on a flat plate will resist spreading and retain a spherical shape, because it tries to minimize its surface area and thus to minimize its surface energy. This surface tension developed at the surface of the liquid results from the attractive forces between molecules of liquid. A pure liquid always tends to reduce its free energy through the action of surface tension, which is quantitatively equal to the amount of work that would be necessary to compensate the natural reduction in free surface energy. In order to enlarge a liquid surface, the bonds between liquid molecules need to be broken.

A large number of soluble materials, such as detergents, can change the surface tension of a liquid. Such materials decrease the tension at the surface during movement of the solute to the surface. A solute that decreases surface tension must be concentrated at the surface because the solvent molecules have a smaller attractive force for the molecules of the solute than for each other. The phenomenon of increased concentration of the soluble material in a boundary or surface is adsorption (Weber, 1972).

2.1.3. Types of Adsorption

There are two primary driving forces for adsorption of a solute from a solution onto a solid phase. The first primary driving force is a consequence of lyophobic (solvent disliking) character of the solute relative to the solvent. The second driving force is a consequence of the high affinity of the solute for the particular solid. Adsorption results from the action of these two forces in most of the wastewater treatment practices.

The degree of solubility of a dissolved substance is the most important factor for the intensity of the two driving forces. A hydrophilic substance is less adsorbable on a solid phase because it likes the solvent system. On the other hand, a hydrophobic substance is water disliking and is more likely to be adsorbed on the adsorbent. Many organic contaminants have amphoteric character with both hydrophobic and hydrophilic groups present in the molecule structure. In this case, the hydrophobic part of the molecule is adsorbed, and the hydrophilic part tends to stay in the solution.

The second primary driving force for adsorption; the affinity of solute for the solid may be due to electrical attraction of the solute to the solid, van der Waals attraction or the chemical interaction with the adsorbent.

Adsorption due to electrical adsorption is a process in which ions in the solution concentrate at the adsorbent surface as a result of electrostatic attraction to charged sites at the surface. This type of adsorption is termed the exchange adsorption. Ion exchange is an exchange adsorption phenomenon. The charge of the ion is the determining factor for exchange adsorption. Ions with the higher charge are preferably adsorbed.

Adsorption occurring as a result of van der Waals forces is generally termed physical adsorption. In physical adsorption, adsorbed molecule is not affixed to a specific site but is free to undergo translational movement within the interface. Physical adsorption is predominant at low temperatures, and is characterized by a relatively low adsorption energy. The adsorbate is less strongly held by the adsorbent compared to chemical adsorption.

Chemical adsorption, or chemisorption, is the phenomenon in which the adsorbate undergoes chemical interaction with the adsorbent. Chemically adsorbed adsorbates are not free to move on the surface or within the interface. Chemical adsorption is characterized by a high adsorption energy, since the adsorbate forms strong localized bonds at active centers on the adsorbent. Chemical adsorption is more predominant at high temperatures, because chemical reactions proceed more rapidly at higher temperatures.

The three forms of adsorption interact in most of the adsorption phenomena. Adsorption processes involving organic molecules are designated as specific adsorptions that exhibit a large range of binding energies from values associated with physical adsorption to higher energies involved in chemical adsorption. Therefore it is generally difficult to distinguish between physical and chemical adsorption in wastewater treatment processes aiming the removal of organic contaminants (Weber, 1972).

2.1.4. Factors Influencing Adsorption

2.1.4.1. Surface Area of Adsorbent. The extent of adsorption is proportional to specific surface area due to the fact that adsorption is a surface phenomenon. Specific surface area is the available portion of the total surface area for adsorption. Therefore, the more finely divided and the more porous the solid, the greater is the amount of adsorption accomplished per unit weight of a solid adsorbent.

2.1.4.2. Nature of the Adsorbate. The degree of solubility of the solute is of primary concern for adsorption equilibria. There is an inverse relationship between the extent of adsorption of a particulate solute and its solubility in the solvent from which the adsorption

occurs. High solubility means stronger solute-solvent bonds than the attraction forces between the solute and the adsorbent.

Polarity of the adsorbate is another important factor. A polar solute is preferably adsorbed by a polar adsorbent, and a nonpolar solute is more easily adsorbed by a nonpolar adsorbent. Dissociation constants of weak acids and bases, e.g. organic acids, also influence the extent of adsorption. Dissociation constants determine the extent of ionization, and hence the adsorption of a particular acid or base is influenced since the adsorption of ionic and molecular forms differ.

2.1.4.3. pH. Many organics form negative ions at high pH values, positive ions at low pH values, and neutral molecules at intermediate pH ranges. Adsorption of many organic materials is higher with neutral orientations. In general, adsorption of organic pollutants by activated carbon from water is increased with decreasing pH. This results from the neutralization of negative charges at the surface of the carbon at low pH values due to high hydrogen concentration. Thereby hindrance of diffusion due to negative charges is reduced and more active sites become available for adsorption. The degree of this effect varies due to the type and activation technique of the active carbon, and the characteristics of the wastewater.

2.1.4.4. Temperature. Since the adsorption reactions are exothermic, the extent of adsorption generally increases with decreasing temperature.

2.1.5. Adsorption Equilibrium and Isotherms

When an adsorbent is in contact with the surrounding fluid of a certain composition, adsorption takes place and after a sufficiently long time, the adsorbent and the surrounding fluid reach a dynamic equilibrium. At this position of equilibrium, there is a defined distribution of solute between the solid and liquid phases. The distribution ratio is a measure of the position of equilibrium in the adsorption process; it may be a function of the concentration of the solute, the concentration and nature of competing solutes, the nature of the solution, and so on.

The relation between amount adsorbed, q, and concentration in the liquid phase, C, at temperature, T, is called the adsorption isotherm at T.

$$q = q(C) \text{ at } T \tag{2.1}$$

The adsorption isotherm is a functional expression for the variation of adsorption with concentration of adsorbate in bulk liquid at constant temperature (Eq. 2.1). The amount of adsorbed material per unit weight of adsorbent increases with increasing concentration, but not in direct proportion (Suzuki, 1990). Adsorption isotherms are described in many mathematical forms, some of which are based on simplified physical picture of adsorption, while others are purely emprical and intended to correlate the experimental data in simple equations. The most widely used mathematical expressions of adsorption equilibrium are Freundlich, Langmuir and BET isotherm expressions.

2.1.6. Activated Carbon

Activated carbons are the microporous carbonaceous adsorbents. A large variety of organic solutes and a more limited number of inorganic solutes can be removed from aqueous waste streams by adsorption onto activated carbon. Activated carbon has a high adsorptive surface area (500-1500 m^2/g) and is used as powdered activated carbon (PAC) or granular activated carbon (GAC) bed. Carbon adsorption process works best with chemicals that have a low water solubility, high molecular weight, low polarity, and low degree of ionization.

2.1.6.1. Preparation of Activated Carbons. Commercially available activated carbons are prepared from carbon-containing source materials such as coal (anthracite or brown coal), lignite, wood, nut shell, petroleum and sometimes synthetic high polymers. These materials are first pyrolized and carbonized at several hundred degrees centigrade. The volatile fraction and low molecular products of hydrolysis are removed during this process, and the residual carbonaceous material undergo the following activation process by using oxidation gases, such as steam at above 800°C or carbon dioxide at higher temperatures. Micropores are formed during the activation process. The yield of activation process is in most cases below 50% and sometimes less than 10%.

2.1.6.2. Activated Carbon Characteristics. Activated carbon is composed of microcrystallites that consist of fused hexagonal rings of carbon atoms with a structure similar to graphite (Yonge et al., 1986). Micropores of activated carbon, where most adsorption takes place, are in the form of two-dimensional spaces between two graphite-like walls, two-dimensional crystalline planes composed of carbon atoms (Suzuki, 1990). The distance between the two neighboring planes of graphite is 0.376 nm, but in the case of activated carbons, which have a rather disordered crystalline structure (turbostratic structure), this distance must be larger since adsorbate molecules are not accessible otherwise (Fig. 2.1). Functional groups that terminate the microcrystallite planes interconnect the microcystalles. Adsorption occurs on the planar surfaces of the microcrystallites and at the functional groups on the edges of the planes. The majority of adsorption that occurs on the microcrystallite planes result from van der Waals forces, while adsorption at the edges of the microcrystallite occurs because of chemical bonding (Yonge et al., 1986).

Activated carbons generally contain some oxygen complexes which arise from either raw materials or from chemical adsorption of air during the activation process or during storage after activation. Surface oxides add a polar nature to activated carbons. Oxygen complexes on the activated carbon surface exist mainly in the form of four different acidic surface oxides; strong carboxylic groups, weak carboxylic groups, phenolic groups, and carbonyl groups. There are several other forms of oxides including basic groups such as cyclic ether groups. The basic character of activated carbon arises when activation is conducted at higher temperatures. Surface oxide groups can be removed by heat treatment of carbons in an inert atmosphere or under vacuum (Suzuki, 1990).

Infrared internal reflectance spectrophotometric techniques enabled substantiation of the presence of significant numbers of carbonyl and carboxyl functional groups on the activated carbon surfaces (Mattson and Mark, 1969). Mattson (1969) showed that *p*-nitrophenol was adsorbed at the carbonyl oxygens on the carbon surface according to a donor-acceptor complexation mechanism. The carbonyl oxygen acted as the electron donor and the aromatic ring of the solute acted as the electron acceptor. To a lesser extent, adsorption occurs by hydrogen bonding of the phenolic protons with surface functional

groups and by complexation with the rings of the microcrystallite planes on exhaustion of the surface functional group (Yonge et al., 1986).

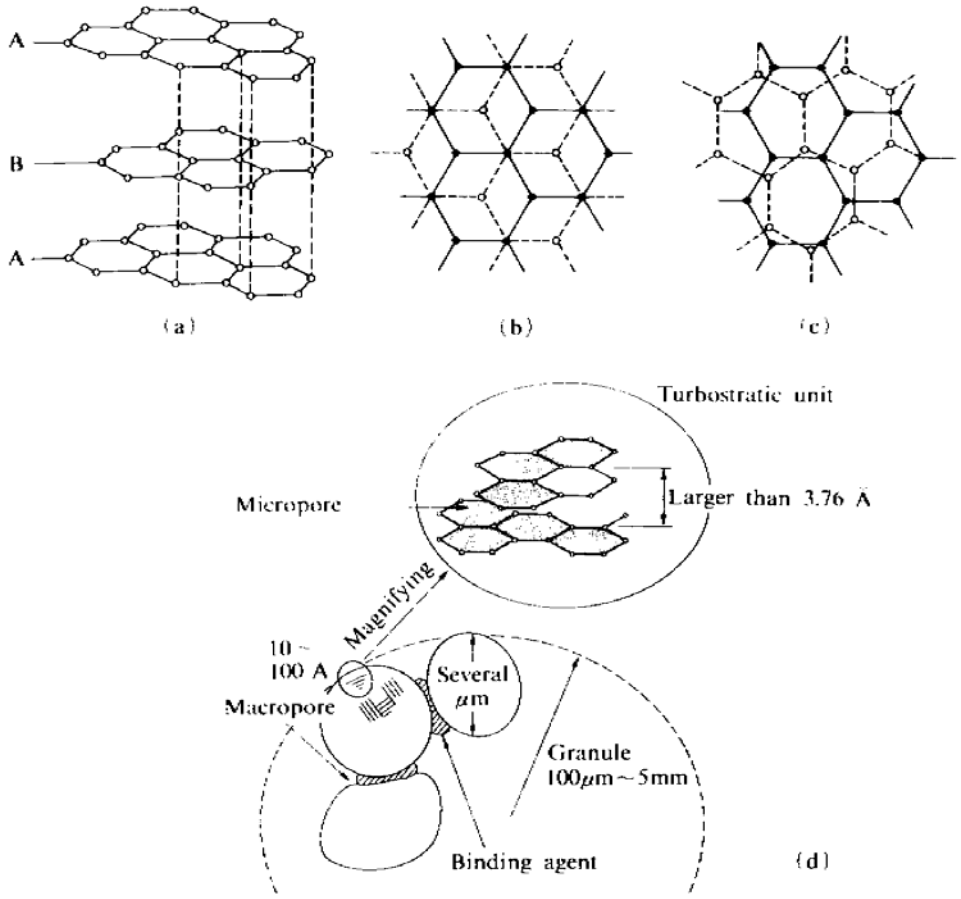


Figure 2.1. Graphite structure (a,b) and turbostratic structure (c) of activated carbon. Conceptual illustration of granular activated carbon (d) (Suzuki, 1990).

Chemical studies have shown that activated carbon may contain large quantities of mineral ions, mostly calcium, sulfate and phosphate ions. These groups influence surface properties in the same way as do acidic or basic organic surface functional groups (Julien et al., 1998).

Activated carbon also contains some ashes derived from the raw material. The amount of ash ranges from 1% to 12%. Ashes consist mainly of silica, alumina, iron, alkaline and alkaline earth metals. The ashes in activated carbon increase hydrophilicity of activated carbon and this is advantageous when PAC is used for water treatment because

the PAC is more likely to stay inside the reactor rather than sticking on the reactor walls if the ash content is high. Another function related with ash content is that the catalytic effects of alkaline-earth and some other metals during activation or regeneration process modifies the pore size distribution to larger pore range.

2.1.6.3. Powdered Activated Carbon. Powdered activated carbon (PAC) is generally produced from wood in the form of saw dust. The average size of PAC is in the range of 15-25 μm. Intraparticular diffusion is not the rate limiting step in PAC adsorption due to the very low particle sizes. PAC is considerably used in water treatment for both drinking water and watewater treatment. PAC is either added to activated sludge or contacted with the wastewater seperately. PAC is also used in chemical industry for decolorization in food processes such as sugar refinery, for oil production and sodium glutamate production as well as wine preparation.

Besides the adsorption characteristics of PAC, its surface charge may also cooperate as a coagulant for colloidal fractions in the liquid phase. Therefore, the regeneration of PAC may become rather difficult, because seperation of colloidal particles will be required before regeneration. Spent PAC is generally dumped rather than regenerated for reuse.

2.1.6.4. Granular Activated Carbon. Granular activated carbons (GAC) are either in the form of crushed granules (coal or shell) or in the pelletized form prepared by granulation of pulverized powders using binders such as coal tar pitch. GAC produced from petroleum pitch is prepared by activation of the spherical beads prepared from the pitch. In liquid phase adsorption, the intraparticle diffusion often becomes the rate limiting step in GAC adsorption. Particle sizes in the range of 12/42 mesh are advantageous for liquid phase adsorption.

GAC filters are widely used in drinking water and wastewater purification processes as an advanced treatment method. In some GAC applications for wastewater treatment, a microbiological film can form on the GAC particles for biological treatment combined with GAC adsorption. GAC is also used in industry for decolorization in sugar refinery. Spent GAC can be regenerated by a thermal method for reuse. Therefore, the consumption of GAC is normally less than powdered activated carbon (Pinker, 1997).

2.2. Biological Treatment of Phenolic Compounds

2.2.1. Biodegradation of Phenolic Compounds

Phenol and its homologues are aromatics containing hydroxyl, methyl, amide, chloride, nitro and sulphonic groups attached to the benzenoid molecules. These molecules are both anthropogenic and xenobiotic. Phenols are toxic to several biochemical reactions. However, biological transformation of phenols to non-toxic compounds exists with specialized microbes, having enzymes of aromatic catabolic pathways (Kumaran and Paruchuri, 1997).

Biological treatment of phenol is usually preferred, because it has the potential to almost completely degrade phenol while producing innocuous end products with minimum secondary waste generation (Goudar et al., 2000). Biodegradation of phenol has been extensively investigated in the literature. Several studies have shown that phenol can be aerobically biodegraded by a wide variety of microorganisms, including pure bacterial cultures (Collins and Daugulis, 1997), mixed bacterial cultures (Rozich and Gaudy, 1985), yeast (Yang and Humphrey, 1975) and filamentous fungi (Garcia et al., 1997).

Various expressions have been proposed for phenol biodegradation, including the Haldane, Monod, zero order, simple first-order and second order models (Lewandowski et al, 1988a). The inhibitory nature of phenol at high concentrations is well known. A variety of substrate inhibition models has been used to describe the dynamics of pure and mixed culture microbial growth on phenol (Goudar et al., 2000). Despite their empirical nature, the substrate inhibition models are able to accurately describe phenol biodegradation data. Haldane type kinetic model is the most extensively used model to describe phenol biodegradation (Magbanua et al., 1994; Goudar et al., 2000). However, the inhibitory concentration is dependent on several factors, such as type of microorganisms and operational conditions. Hence, a very broad range of kinetic parameters were reported in literature (Magbanua et al., 1994; Kumaran and Paruchuri, 1997; Buitron et al., 1998; Goudar et al., 2000).

In literature, there are various aerobic and anaerobic treatability studies on chlorophenols (Sahinkaya and Dilek, 2002). Puhakka et al. (1992) achieved degradation of high concentrations of various chlorophenols in anoxic denitrifying biofilm systems as well as in aerobic fluidized-bed reactors. However, only trace concentrations of chlorophenols could be removed in conventional municipal sewage treatment plants (Ettala et al., 1992).

It has been shown that halogenated phenols can be resistant to an aerobic degradation, because the benzene ring becomes deactivated to the electrophilic attack of oxygen by withdrawing the electrons from the ring (Fahmy et al., 1994). The chlorinated compounds seemed to undergo a preferential *o*-methylation, and this served as a detoxification mechanism. Under anaerobic conditions, chlorophenols undergo a reductive dechlorination process with the chlorine atom being replaced by a hydrogen atom. Dechlorination preferentially takes place in the ortho-position, resulting in a sequence of removal rates for monochlorophenols as ortho>meta>para (Fahmy et al., 1994). For example, 2,4-dichlorophenol can be completely degraded to 4-chlorophenol and phenol sequentially, although 4-chlorophenol may build-up and cause inhibition (Zhang and Wiegel, 1990).

The growth of microorganisms in the presence of multiple phenolic compounds were investigated by several researchers (Yu and Hu, 1994; Bae et al., 1995; Buitron et al., 1998; Quan et al., 2005). Bae et al. (1995) found out that although phenol was completely biodegraded, addition of dichlorophenol and pentachlorophenol resulted in incomplete biodegradation of all phenolic compounds. The long-term exposure of a mixed culture to chlorinated phenols resulted in a decrease in growth rates and increased substrate inhibition, which was probably caused by a loss in bacterial species diversity (Bae et al., 1995).

Acclimation of mixed bacterial cultures to phenolic compounds successfully increases the biodegradability of these compounds (Buitron et al., 2005). Buitron et al. (1998) showed that an acclimated activated sludge could degrade a mixture of phenol and several chlorophenols by one or two orders of magnitude faster than pure strains obtained from the acclimated consortium. Lewandowki et al. (1988b) also showed that both phenol

and 2-chlorophenol were biodegraded much more readily with phenol-acclimated microorganisms rather than non-acclimated ones. Wiggings et al. (1987) suggested that there is a selection and multiplication of specialized microorganisms during the acclimation phase. However, the recalcitrant property of some chlorophenolic compounds was not always easy to alter through biological acclimation because microorganisms acclimated to one chlorophenolic compound did not necessarily gain access to the other (Yu and Hu, 1994).

On the other hand, bioaugmentation of microorganisms is generally effective for one target pollutant or a group of pollutants. Therefore, bioaugmentation in the treatment of phenolic compounds does not always work (Quan, et al., 2005). Besides the target compounds, other compounds in wastewaters may be toxic and inhibitory to the special culture augmented. However, in some cases bioaugmentation can also be successfully applied. In the study of Quan et al. (2005), an activated sludge system bioaugmented with 2,4-dichlorophenol degrading special culture could degrade both 2,4-dichlorophenol and 4-chlorophenol. Lewandowski et al. (1988a) isolated eleven species from a phenol-acclimated activated sludge. Only three of these species could biodegrade phenol, when they were isolated. However, these species could not degrade phenol, when they were purchased from commercial suppliers and used without acclimation. The study of Lewandowski et al. (1988a) showed that use of acclimated mixed cultures is more efficient than isolated pure cultures. Frequently, a single aerobic species can totally mineralize chlorinated phenols (Sahinkaya and Dilek, 2002). However, anaerobic degradation of chlorophenols requires more than one organism for complete degradation. For example, at least five groups of organisms interacting sequentially were required for the complete degradation of 2,4-dichlorophenol under anaerobic conditions (Zhang and Wiegel, 1990).

2.2.2. Cometabolic Degradation of Inhibitory Phenolic Compounds

Biological treatment of wastewaters or industrial effluents often involves utilization and transformation of mixed substrates. Toxic compounds (both growth and non-growth substrates) and non-toxic, easily biodegradable compounds often co-exist in effluents. Substrate interactions among the multiple substrates are often quite complicated and may result in sequential or simultaneous utilization. Some growth substrates (such as phenol)

and non-growth substrates (such as chlorophenols) exert substrate toxicity on cell growth as well as on degradation capacity at high concentrations. For biotransformation of non-growth substrates, cometabolism is encountered (Wang and Loh, 2000). For example, 2-chlorophenol biodegradation could be successfully enhanced in the presence of growth substrates such as dextrose and phenol (Basu and Olezkiewicz, 1995). Other mono-chlorophenols were also degraded by a cometabolic mechanism in the presence of phenol by an acclimated pure culture (Kim and Hao, 1999).

Cometabolism is the biological transformation of a non-growth substrate by bacteria through the catalysis of nonspecific enzymes. Synthesis of these enzymes in microbial cell can only be induced by a growth-substrate, which provides energy for cell growth and maintenance of bacteria. Although cometabolism involves the simultaneous transformation of growth and non-growth substrates, microorganisms cannot derive cell carbon or energy benefit from transformation of the non-growth substrate and hence cannot induce production of enzymes (Wang and Loh, 2000). Therefore, a growth-substrate must be available at least periodically to grow new cells and to induce production of the enzymes necessary for cometabolic transformation of non-growth substrate (Alvarez-Cohen and Speitel, 2001). Cometabolism can be accomplished by growing cells in the presence of a growth substrate, resting cells in the absence of a growth substrate, and resting cells in the presence of an energy substrate. The energy substrate is an electron donor that provides reducing power and energy, but does not support growth by itself (Criddle, 1993).

Simultaneous utilization of multiple substrates is commonly observed in wastewater treatment systems. For example, cell growth on mixtures of solvents and chlorinated aromatic compounds displayed concurrent substrate utilization (Wang and Loh, 2000). Sequential utilization is much rare in the biodegradation of hazardous compounds. In the study of Ampe et al. (1998), organic acid metabolism completely blocked phenol catabolism resulting in sequential utilization. In general, simultaneous utilization of mixed substrates is much more desirable than sequential utilization, since enhanced degradation efficiencies are usually accomplished in the former. However, degradation of one growth substrate is severely inhibited by the presence of the other in sequential utilization. Therefore, a long lag or acclimation phase is required for the utilization of the second

substrate (Wang and Loh, 2000). Sequential utilization pattern was reported in the degradation of mixed chlorinated phenols (Menke and Rehm, 1992).

However, biodegradation pattern of mixed substrates may not necessarily fall into one of the two distinctive behaviours of simultaneous or sequential utilization. Wang and Loh (2000) suggested a new cell growth pattern characterized by two exponential growth phases separated by an intermediate lag phase. In that study, phenol was a specific growth substrate, which easily induced necessary enzymes for the cometabolic transformation of 4-chlorophenol.

Cometabolism may result in biodegradation of slowly biodegradable or nonbiodegradable compounds. However, cometabolism inherently tends to be an unsustainable process. For example, the presence of a non-growth substrate can inhibit metabolism of the natural growth-substrate, thereby decreasing or preventing bacterial growth. In addition, cometabolic transformations consume reductant (e.g., NADH) that otherwise would be available to support metabolism of the growth-substrate. Moreover, cometabolic transformation of some non-growth substrates can lead to inactivation of bacteria by damaging important cellular constituents. However, cells can recover from cometabolism associated injuries, even in the presence of the injury-inducing non-growth substrate (Ely et al., 1997).

The cometabolic biotransformations of non-growth substrates are catalyzed by non-specific enzymes induced by growth substrates. Enzymes are organic catalysts, globular protein molecules that are synthesized in the cytoplasm and speed up chemical reactions. They achieve this by this by forming an enzyme-substrate complex, which lowers the energy of activation that must be supplied to cause molecules to react with each other (Kim and Hao, 1999).

Non-growth substrates that have a similar structure to that of a growth substrate could bind to enzymes and be transformed due to non-specific activity. Particularly, oxidative enzymes (e.g. oxygenases) responsible for the oxidation of phenols, ammonia and methane by various microorganisms have shown non-specific activities toward non-growth substrates such as chlorinated aromatic compounds (Kim and Hao, 1999). Resting

cells of these cultures have been frequently used for the degradation of toxic pollutants. However, the efficacy of resting cell transformation is limited by the depletion of cells internal energy electron supply and product toxicity (Kim and Hao, 1999).

The aerobic cometabolic transformation of chlorinated organics (Figure 2.2) are catalyzed by nonspecific oxygenase enzymes that use molecular oxygen as the electron acceptor and NAD(P)H as the reducing energy (electron) donor to oxidize both growth-substrates and cometabolic (non-growth) substrates (Chang and Alvarez-Cohen, 1995). Oxygenases constitute a subset of the enzymes classified as oxidoreductases. Oxygenases catalyze the reduction of O_2 with incorporation of one (monooxygenases) or two (dioxygenases) of the O atoms into the substrate that is being oxidized. Any organism with an active mono- or di-oxygenase has the potential for cometabolic transformation (Kocamemi, 2005). However, some factors may adversely affect the cometabolic degradation by oxygenase-expressing microorganisms. These factors include product toxicity, enzyme inhibition by growth or other cometabolic substrates and reducing energy or reductant shortages (Alvarez-Cohen and Speitel, 2001).

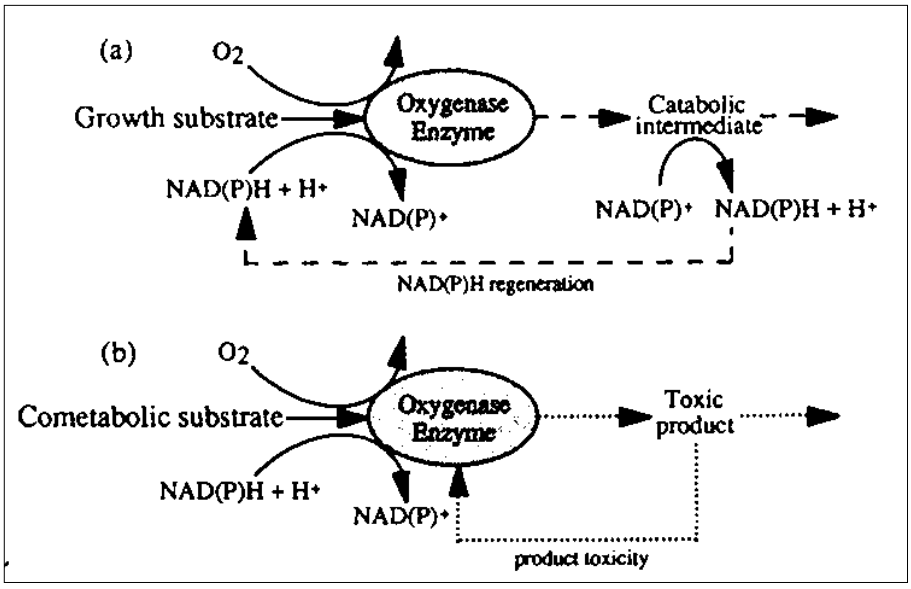


Figure 2.2. Typical oxygenase enzyme reactions for growth-substrate and cometabolic (non-growth) substrate (Chang and Alvarez-Cohen, 1995).

2.3. Bioregeneration of Activated Carbon

2.3.1. Enhancement of Biological Treatment by Activated Carbon Adsorption

There is considerable evidence in literature about the advantages gained by adding powdered activated carbon (PAC) to activated sludge systems. It is reported that this powdered activated carbon treatment (PACT) helps stabilize the process through improved sludge settling, improved sludge thickening/dewatering, increased organics removal, improved removal of refractory and inhibitory organics, reduction of the impact of organic shock loadings, increase of contact time between biomass and activated carbon, increase of organic carbon and oxygen concentration at the surface of activated carbon, increased color removal, suppressed aerator foaming, improved nitrification, increased solid residence times, and reduction in odor and sludge bulking over conventional systems (Aktaş, 1999; Çeçen and Aktaş, 2003; Çeçen and Aktaş, 2004; DeWalle and Chian, 1977; Sublette et al., 1982; Ng et al., 1987; Kim et al., 1997; Kim et al., 1998; Lim et al., 2002). In Biological Activated Carbon (BAC) filters, biological treatability is enhanced through the adsorption of inhibitory substances as in the PACT process and also the granular activated carbon (GAC) used in these filters serves as a supporting medium for the biofilm (Imai et al., 1995).

Organic matter removal in a PACT or BAC system is the combination of adsorption and biodegradation. Activated carbon, when used in conjunction with biological treatment gives higher organic removal efficiency than separate adsorption and biological treatment. Many researchers suggest that a synergy exists between activated carbon and microorganism activities (Andrews and Tien, 1982; Sublette et al., 1982; Olmstead and Weber, 1991; Orshansky and Narkis, 1997; Seo et al., 1997; Sirotkin et al., 2001). A combined system can remove an organic compound more efficiently than expected from either biodegradation or adsorption alone. The mechanism consists of the stimulation of biological activity due to adsorption of inhibitory substances and bioregeneration of activated carbon by microorganisms for further adsorption. Activated carbon provides an attachment surface for microorganisms and protects them from shock loadings of toxic and inhibitory materials, whereas microorganisms bioregenerate the activated carbon.

Stimulation of biological activity is generally the main mechanism in the enhancement of organic matter removal in PACT and BAC systems (Sublette et al., 1982).

Activated carbon is widely used for the removal of the total parameters BOD and COD, and the removal of specific aromatic compounds, aliphatics and halogenated aliphatics. In general, activated carbon adsorption processes work best with organics that have a low water solubility and a high molecular weight. Therefore, a compound such as methanol can be only poorly adsorbed while chlorinated aromatic compounds are very well adsorbed. Wastewaters having a high BOD generally exhibit less adsorption, because BOD represents rather the low molecular weight compounds. On the other hand, activated carbon is more suited to the removal of nonbiodegradable COD (Sublette et al., 1982; Imai et al., 1995).

However, preferential adsorption of high molecular weight organics is not always the case, because activated carbon can hardly adsorb organics with very high molecular weights (HMW). It is suggested that activated carbon preferentially adsorbs refractory organics with lower molecular weights (LMW). However, this does not also mean that activated carbon preferentially adsorbs very low molecular weight organics that are more readily biodegradable. The LMW refractory organics in wastewaters are also more biodegradable than the HMW refractory organics in the case of bacterial acclimation and bioregeneration. Therefore, microorganisms also prefer LMW refractory organics for biodegradation during the bioregeneration of activated carbon (Imai et al., 1995).

Besides being very effective with regard to adsorption capacity, PAC may also provide an attachment surface for microorganisms (Ng et al., 1987). In particular, biological treatment of industrial wastewater has been reported to be efficient in immobilized systems using synthetic or natural carriers such as activated carbon (Abu-Salah et al., 1996). In activated sludge processes with PAC addition, the property of providing an attachment surface for microorganisms is less possible than in BAC processes. In fixed film or in fluidized bed BAC processes, a biofilm forms on the attached media (GAC). Since in attached growth processes the solids retention time is generally much higher than in activated sludge processes, a sufficient time is provided for microorganisms to attach on activated carbon (GAC) surfaces. In an activated sludge

process, microorganisms may not have enough time to attach on PAC surfaces because of lower sludge age. Therefore, attachment efficiency is much lower in PAC added activated sludge systems compared to biologically activated GAC filter systems (Imai et al., 1995).

However, when activated carbon is used in a suspended medium, the use of PAC is more advantageous than GAC since PAC systems provide a uniform distribution of solids with a minimum energy requirement for mixing and arrive at adsorption equilibrium in a relatively short period of time. Regeneration technologies minimize the disposal problems associated with PAC and effective clarification in the presence of PAC makes continuous operation possible in some cases.

Many studies in the literature showed that nitrification was enhanced by PAC addition to activated sludge (Ng and Stenstrom, 1987; Ng et al., 1987; Aktaş and Çeçen, 2001a; Aktaş and Çeçen, 2001b; Çeçen and Aktaş, 2001a). Studies have demonstrated that ammonia removal is significantly enhanced by the addition of PAC. The reason for enhanced nitrification is the removal of toxic and inhibitory organics or inorganics by PAC. This enhancement in nitrification is more pronounced compared to the enhancement in organic carbon removal, because nitrifiers are more sensitive to inhibitors than heterotrophes (Ng et al., 1987; Çeçen and Aktaş, 2001a).

2.3.2. Bioregeneration Theories

Bioregeneration is a term used to describe the synergism between activated carbon and microorganisms in a combined system of activated carbon and biological treatment. It is the renewal of carbon's adsorptive capacity by the activity of microorganisms so that further adsorption can take place. Bioregeneration can be achieved either by mixing bacteria with saturated activated carbon in offline systems (Goeddertz et al., 1988; Holst et al., 1991; Scholtz and Martin; 1997; Roy et al., 1999; Silva et al., 2004) or by simultaneous treatment as in the case of PACT (powdered activated carbon assisted activated sludge treatment) or BAC (Biological activated carbon) systems (Sublette et al., 1982; Speitel and DiGiano, 1987; Jonge et al., 1996a; Ha and Vinitnantharat, 2000; Vinitnantharat et al., 2001). Bioregeneration can be optimized by varying the nature of microorganisms, environmental conditions and loading on activated carbon (Vinitnantharat et al., 2001).

Offline bioregeneration employs desorption and biological activity in a closed loop recirculating batch system. Offline bioregeneration includes taking exhausted activated carbon out of service, and regenerating by recycling a mixture of acclimated bacteria, nutrients, and dissolved oxygen through the activated carbon column in an environment favourable to enhance biodegradation of adsorbed organic matter (Goeddertz et al., 1988). Goeddertz et al. (1988) suggested that the offline bioregeneration process for activated carbon could be more generally applied compared with the simultaneous BAC treatment based on the limitations of the BAC process, such as availability of nutrients and dissolved oxygen, persistence of many organic compounds and operational difficulties including hydraulic short-circuiting and excessive head loss. Kolb and Wilderer (1997) reported that an offline BAC bioregeneration system led to higher degradation rates than simultaneous BAC operation. Hutchinson and Robinson (1990a, 1990b) developed an offline GAC bioregeneration by recirculation of microorganism-free filtered permeate through the GAC column and a separate bioreactor. This bioregeneration process was superior to the recirculation of microorganisms through the column without pre-filtration of the microorganisms, because microbial fouling was prevented in the GAC reactor (Hutchinson and Robinson, 1990b).

Several researchers state that activated carbon is bioregenerated in PACT processes (Sublette et al., 1982; Schultz and Keinath, 1984; Chudyk and Snoeyink, 1984; Kim et al., 1997; Seo et al., 1997). But, there are also studies showing that the bioregeneration hypothesis is not always valid and in some cases the PACT process is a simple combination of adsorption and biodegradation (Xiaojian et al., 1991; Çeçen, 1994; Bornhardt et al., 1997; Garner et al., 2001).

The regeneration of activated carbon by a bacterial biofilm in BAC (biological activated carbon) systems has also been reported by several authors (Imai et al., 1995; Ha et al., 2000; Vinitnantharat et al., 2001; Badriyha et al., 2003). BAC systems involve granular activated carbon (GAC) particles covered by biofilm and are usually applied in fluidized bed reactors. Sirotkin et al. (2001) separated the processes in BAC filtration into three phases. The initial phase consisted of the preferred adsorption of contaminants from wastewater when the adsorption rate considerably surpassed the biodegradation rate. The second phase was the primary adsorption equilibrium when the rate of adsorption and

biodegradation were comparable. The third phase consisted of dynamic conditions when the biodegradation rate could be higher than the adsorption rate, and desorption from the pores might occur resulting in regeneration of carbon. However, in BAC systems biofouling can occur due to excessive growth of microorganisms and therefore biofouling and bioregeneration may be two mechanisms opposing each other (Vuoriranta and Remo, 1994; Scholz and Martin, 1997).

Proposed theories of bioregeneration require that a compound should be initially adsorbed onto the carbon's surface where microorganisms reside, and that adsorption consequently results in higher substrate concentrations at the surface than the bulk. This increase in concentration stimulates the biological growth on carbon surface and replenishes activated carbon for further adsorption. The bioregeneration mechanism includes desorption of the substrate and subsequent biodegradation, which depends on a number of factors such as the nature of microbial population, adsorbability and reversibility of adsorbate (Vinitnantharat et al., 2001).

In literature two mechanisms are mentioned leading to bioregeneration of activated carbon. One of them is biodegradation of target compounds following desorption due to a concentration gradient between the activated carbon surface and bulk liquid. According to this theory, on the surface of carbon the adsorption concentration rises more than the equilibrium adsorption concentration through the decrease of the bulk concentration by biological activity, and the adsorbed organic matters are desorbed due to the concentration gradient between the activated carbon surface and bulk liquid (Schultz and Keinath, 1984; Jonge et al., 1996a; Kim et al., 1997). Besides the concentration gradient, the difference in the Gibbs free energy between the adsorbate molecules in solution ($-\Delta G°_{ads}$) and the modified adsorbate molecules inside the porous structure ($-\Delta G°_{mod}$) was suggested to be a driving force for bioregeneration (Klimenko et al., 2002).

The second theory includes desorption of organic matter according to exoenzymatic reactions. Some researchers stated that the bioregeneration mechanism involved extracellular enzymes (Perotti and Rodman, 1974; Kim et al., 1997; Sirotkin et al., 2001). Others indicated a mechanism involving desorption due to a concentration gradient only and stated that nondesorbable compounds could not be bioregenerated (Schultz and

Keinath, 1984; Speitel et al., 1989a; Olmstead and Weber, 1991; Jonge et al., 1996a). According to the theory including exoenzymes, some enzymes excreted by bacteria could easily diffuse into pores and react with adsorbed substrates. Then, hydrolytic decay of the substrate may occur or further desorption may take place due to the weak adsorbability of organic matter reacting with the enzyme. Li and DiGiano (1983) suggested that these extracellular enzymes would be adsorbed before they could act.

On the other hand, it is also suggested that enzyme molecules are larger than the sizes of micropores, so that bioregeneration cannot occur due to enzymatic reactions (Xiaojian *et al*, 1991). It was stated that the diameter of the pore in which the exoenzyme can catalyze should be larger than 10 nm (Xiaojian *et.al*., 1991), which does not include the micropores (Φ<2nm) and some of the mesopores (Φ 2-50nm) Therefore, the effect of hypothetical exoenzymatic reactions is expected to be limited for low molecular weight organics. Other researchers also state that the micropores of activated carbon are occupied by adsorbed molecules and are not subject to bioregeneration (Klimenko et al., 2002). However, there are also studies indicating that a biofilm can be established on the BAC, which contains bacteria in the macropores and their exoenzymes in the micropores (Scholtz and Martin, 1997).

Some researchers support the idea that exoenzymatic activity is possible in meso- and macropores (Sirotkin et al., 2001; Klimenko et al., 2003). However, Xiaojian et al. (1991) state that no evidence exists that exoenzymes are involved in ring hydroxylation and ring fission reactions of aromatic compounds and phenols. Thereby, they propose that the bioregeneration hypothesis including exoenzymatic reactions is not valid for these compounds. Previously, Benedek et al. (1980) argued that extracellular enzymes were probably incapable of breaking down small molecules and that degradation in pore volumes was insignificant. Andrews and Tien (1975) also suggested that bioregeneration mechanisms including enzymes would be extremely slow due to low diffusivity of large hydrolytic enzymes within the pores.

In the study of Ha and Vinitnantharat (2000), bioregeneration efficiencies exceeded the total desorbabilities. Although not stated by Ha and Vinitnantharat (2000), bioregeneration did not only occur due to the concentration gradient, but exoenzymatic

reactions were probably responsible for that unexpectedly high bioregeneration efficiencies. Sirotkin et al. (2001) suggested that desorption from micropores took place due to a reverse concentration gradient, and desorption from mesopores was also supported by the activity of exoenzymes. The authors concluded that the process of bioregeneration was featured by the two non-contradictory hypotheses. However, bioregeneration due to exoenzymatic reactions is still a hypothesis and the mechanisms need to be further investigated.

Adsorption-induced acclimation of microorganisms is another mechanism proposed for bioregeneration (Maloney et al., 1983; Ng and Stenstrom, 1987; Imai et al., 1995). The adsorption of refractory organics on the activated carbon, which is in intimate contact with microorganisms, induces the acclimation of attached microorganisms to these compounds. This idea differs from the previous ones in the way that the nature of bacterial culture changes in order to use refractory organics as substrates. This suggestion does not seem to be valid for PAC added activated sludge systems at low sludge ages. The hypothesis of bioregeneration is more likely to be valid in nitrification systems operating at higher sludge ages than those primarily aiming at carbonaceous BOD removal. However, in the study of Maloney et al. (1983), acclimation of bacteria to adsorbed substrate was not a significant mechanism in BAC filtration of potable water, and the authors concluded that carbon regeneration might be assisted by bacteria, but was not caused by bacteria.

Bioregeneration is also reported in anaerobic biological processes combined with activated carbon addition (Kim et al., 1986; Suidan et al., 1980). Suidan et al. (1980) noted that the carbon equivalent of the gaseous products (methane and carbon dioxide) exceeded the organic carbon removal and stated that the extra gaseous products were due to bioregeneration in an anaerobic BAC system treating catechol. Bioregeneration was also investigated by Sison et al. (1996) and Kim et al. (1997) under the anoxic conditions of a denitrifying BAC filter, which was supplied with sucrose as the organic carbon source. In those studies, BAC served as a storage tank for organic carbon and supplied sucrose to denitrifying bacteria when organic carbon was deficient in the bulk liquid. This process resulted in bioregeneration of loaded activated carbon and the authors (Kim et al., 1997) suggested that bioregeneration was dependent on the carbon/nitrogen ratio in the environment surrounding BAC.

2.3.3. Dependency of Bioregeneration on Reversibility of Adsorption

Desorption from activated carbon occurs in response to changes in operating conditions (Thacker et al., 1983) such as a decrease in the liquid phase concentration, displacement of adsorbed solutes by competitive adsorption and some other changes (such as pH) in the liquid phase decreasing adsorbability. As discussed in the previous section, desorption is a prerequisite step for bioregeneration. Thus, any condition that affects desorption of the adsorbed solute eventually affects the extent of bioregeneration.

In literature it was stated that bioregeneration could only occur with compounds that readily desorb (Walker and Weatherley, 1997). Bioregeneration seems to be limited in the case of some complex substrates, because complex substrates are likely to be irreversibly adsorbed. Hence, bioregeneration is controlled by the reversibility of adsorption (Schultz and Keinath, 1984; Jonge et al., 1996a). When activated carbon was loaded with non-desorbable compounds, it could not be bioregenerated (Schultz and Keinath, 1984; Speitel et al., 1989a; Olmstead and Weber, 1991). Such apparent irreversibility is commonly referred to as hysteresis or nonsingularity (Ha and Vinitnantharat, 2000).

There are two possible mechanisms leading to irreversible adsorption. One of them is the high energy bonding of adsorbate molecules to specific functional groups on the active sites of carbon surface resulting in covalent bonding (Yonge et al., 1985; Ha and Vinitnantharat, 2000; Jonge et al., 1996b). Depending on type of surface functional group and sorbate, a sufficiently strong bond can be formed resisting desorption. Therefore, chemisorption appears to be the most logical explanation for irreversible adsorption, the degree of which is directly related to the number of high-energy (chemisorptive) bonds (Yonge et al., 1985). On the other hand, reversible adsorption was attributed to adsorption as a result of van der Waals forces and/or weaker charge-transfer complexes that occur at adsorption sites (Yonge et al., 1985).

Second is the oxidative polymerization of phenolic compounds onto activated carbon due to the presence of oxygen (Jonge et al., 1996b; Vinitnantharat *et al,* 2001). Phenol molecules that may undergo an oxidative coupling reaction may be irreversibly adsorbed on activated carbon (Vidic et al., 1993; Garner et al., 2001), which in turn may result in a

low bioregeneration efficiency. Phenoxy radicals formed by the removal of a hydrogen atom from each phenolic molecule can participate in direct coupling with other phenoxy radicals at room temperature, activated carbon surface serving as a catalyst (Vidic et al., 1993). Electron localization in the radicals determines the coupling position (ortho or para position to hydroxyl group). Coupling is predominantly achieved through carbon-carbon bonding and less frequently through carbon-oxygen bonding. Molecular oxygen can act as an initiator in coupling reactions by directly reacting with phenol (Vidic et al., 1993) as in Eqn. 2.2.

$$PhOH + O_2 \rightarrow PhO^* + HO_2^* \quad (2.2)$$

In addition, the phenolate ion can also react with oxygen:

$$\begin{aligned} PhOH &\Leftrightarrow PhO^- + H^+ \\ PhO^- + O_2 &\rightarrow PhO^* + HO_2^* \\ O_2^- + H^+ &\rightarrow HO_2^* \end{aligned} \quad (2.3)$$

The radicals formed through equations 2.2 and 2.3 can react with another phenol molecule:

$$PhOH + HO_2^* \rightarrow PhO^* + H_2O_2 \quad (2.4)$$

Hydrogen peroxide reacts with another phenol molecule according to the following equations:

$$PhOH + H_2O_2 \rightarrow PhO^* + H_2O + HO^* \quad (2.5)$$

$$PhOH + HO^* \rightarrow PhO^* + H_2O \quad (2.6)$$

The above reactions were demonstrated to take place at elevated temperatures (180-210°C) and pressures (35 atm), indicating a high activation energy of radical formation. The results of Vidic et al. (1993) indicated that such reactions were also feasible at room temperatures with the activated carbon surface serving as a catalyst.

Another study (Jonge et al., 1996b) demonstrated that adsorption of a phenolic compound, o-cresol, was increased in the presence of molecular oxygen due to oxidative polymerization, whereas adsorption of a nonphenolic compound, 3-chlorobenzoic acid, was not affected by the presence of oxygen. In general, oxidative coupling of phenolic compounds, except nitrophenols, decreased the reversibility of adsorption (Vidic et al., 1993). However, the presence of oxygen had no effect on the adsorptive capacity of mono-nitrophenols. Also, irreversible adsorption was absent for nitrophenols under oxic conditions (Vidic et al., 1993). The addition of electron-withdrawing functional groups like NO_2 increases the potential required to oxidize the organic compound. Among the 35 phenolic compounds tested, nitrophenols had the highest bond dissociation energies for the O-H bond. Vidic et al. (1993) stated that this reversible adsorption behaviour of nitrophenols could be explained by the fact that the catalyzing effect of activated carbon was not strong enough to catalyze oxidative coupling reactions in the case of nitrophenols.

Oxidative coupling of phenolic compounds is also dependent on carbon activation type. In several studies, a higher irreversible adsorption and lower bioregeneration were observed in the case of thermally activated carbons (Jonge et al., 1996a; Jonge et al., 1996b). This was explained by the affinity of thermally activated carbons towards oxygen and changes in surface chemistry upon contact with oxygen (Jonge et al., 1996b). Contrary to this, chemically activated carbons have a surface with fully oxidized active sites so that interaction with oxygen does not affect the surface, whereas thermal activation of the carbons is originally carried out in the absence of oxygen leading to a more reactive surface.

In the study of Jonge et al. (1996b), it was stated that functional groups influence the probability of the sorbate being oxidized. In general, unsaturated groups (e.g. carboxyl and nitro groups) decrease the susceptibility to oxidation, whereas saturated groups (e.g. methyl groups) increase the probability of oxidation. Also in the study of Vinitnantharat et al. (2001), it was hypothesized that phenol and 2,4-dichlorophenol undergo oxidative coupling on the GAC surface and availability of oxygen promotes irreversible adsorption. Adsorption of phenolic compounds was not fully of physical type. However, in literature oxidative polymerization was not reported for nonphenolic compounds.

Adsorption energy is particularly important from the point of adsorption reversibility and bioregeneration. The higher the change in Gibbs free energy of adsorption ($-\Delta G°_{ads}$) of a substrate, the lower bioregeneration was observed in the study of Klimenko et al. (2002). This can be attributed to the high energy of adsorptive binding with the sorbent surface that results in a lower reversible adsorption. Orshansky and Narkis (1997) reported that aniline was not available for biodegradation in the bulk liquid since it was adsorbed on PAC with a higher energy compared to phenol. Orshansky and Narkis (1997) suggested that the energy of adsorption might provide an information on which organic compound could be conveniently removed by simultaneous adsorption and biodegradation.

From the point of water pollution control, ideally adsorption should be irreversible for nonbiodegradable compounds, otherwise desorption would lead to an undesired leakage of compounds to receiving waters. Special attention should be paid to the possible desorption of an already adsorbed organic matter if it is hardly biodegradable such as 4-nitrophenol in the study of Ivancev-Tumbas et al. (1998). However, for biodegradable compounds, desorption will lead to bioregeneration and a renewal of the adsorptive capacity of activated carbon. Irreversible adsorption of biodegradable compounds will unnecessarily deteriorate the adsorptive potential and shorten the service life of activated carbon (Jonge et al., 1996b).

2.3.4. Factors Affecting Bioregeneration

In literature it was suggested that for an effective bioregeneration to take place certain microbiological and technological preconditions were required such as the presence of microbiological agents capable of utilizing adsorbate, presence of mineral components (nitrogen, phosphorus, sulfur, etc.), creation of optimum conditions for the vitality of microorganisms (temperature, dissolved oxygen concentration, etc) and optimization of the proportion between the microorganism and the adsorbate concentrations (Klimenko et al., 2003). Sirotkin et al. (2002) suggested adsorption-desorption balance, residence time and spatial distribution of molecules in carbon pores as the other factors determining the efficiency of bioregeneration. Putz et al. (2005) added the loading on carbon, the adsorption characteristics (i.e. Freundlich K and 1/n) and the location within the GAC column as factors affecting the rate and extent of bioregeneration.

2.3.4.1. Biodegradability. The bioregeneration hypothesis is generally valid for slowly biodegradable and adsorbable organic compounds. Slowly biodegradable compounds can be biodegraded if a sufficient contact time with biomass is provided. In PACT systems, these compounds are in contact with the biomass for a length of time equal to the sludge age if they are adsorbed by PAC and thus enter the solid phase (DeWalle and Chian, 1977). Several compounds, which are considered nonbiodegradable (such as chloroform and chlorinated benzene), were adsorbed on activated carbon and subsequently metabolized by attached microorganisms (Kim et al., 1986). Therefore, bioregeneration should increase with sludge age (Sublette et al., 1982). Further desorption of slowly biodegradable organics shows that bioregeneration of PAC promotes the extent of biodegradation. Hence, it can be said that adsorption acts as a prerequisite step for biodegradation.

On the other hand, readily biodegradable organic matter is generally not adsorbable and can be removed by biological activity alone whereas the removal of nonbiodegradable and adsorbable compounds takes place through simple adsorption. However, Olmstead and Weber (1991) stated that bioregeneration was due to simple desorption of sorbed compounds followed by biodegradation, and hence bioregeneration could only occur in the case of compounds that are both biodegradable and adsorbable. Xiaojian et al. (1991) suggested that bioregeneration was not valid in the case of biodegradable compounds such as phenol during the simultaneous BAC process. The results of other studies indicated that the concept of bioregeneration could be applied to both readily biodegradable and less-biodegradable compounds or to toxic compounds (Ha and Vinitnantharat, 2000; Kim et al., 1986)

2.3.4.2. Substrate Type. In the study of Jonge et al. (1996a), it was shown that the extent of bioregeneration was also dependent on the compound adsorbed. They found that the non-phenolic compound, 3-chlorobenzoic acid, was more available for bioregeneration of PAC compared to the phenolic compound, o-cresol. In the study of Klimenko et al. (2002), phenol with a smaller Gibbs free energy of adsorption ($-\Delta G°_{ads}$) was found to be more suitable for bioregeneration than nonionic and anioic surfactants. Another study (Lee and Lim, 2005) showed that the extent of bioregeneration was greater for phenol-loaded carbon

compared to p-methylphenol-, p-ethylphenol- and p-isopropylphenol-loaded ones. Carbons loaded with alkyl-substituted phenols exhibited less bioregenerability and bioregenerability decreased with an increase in the length of the alkyl chain (Lee and Lim, 2005). In the study of Tanthapanichakoon et al. (2005), aromatic compounds with electron-donating substituent groups (e.g. phenol with a hydroxyl group) exhibited higher irreversible adsorption compared to aromatic compounds with electron-attracting substituent groups. Ha et al. (2001) and Vinitnantharat et al. (2001) also reported that the bioregeneration extent of GAC was higher with phenol compared to the halogen substituted 2,4-dichlorophenol. In the study of Speitel et al. (1987), desorption and bioregeneration were found to be more rapid for p-nitrophenol compared to phenol. Although not stated by the authors, this finding may be due to irreversible adsorption of phenol caused by oxidative coupling whereas oxidative coupling does not occur with nitrophenols (Vidic et al., 1993).

2.3.4.3. Carbon Particle Size. The validity of the bioregeneration hypothesis seems to be higher in biologically activated GAC filters than in PAC added activated sludge processes. The first reason is that the sludge age is much higher in attached growth processes than in activated sludge, and microorganisms often have enough time to degrade adsorbed refractory organisms. The second reason, which is also related to the first one, is that GAC provides an intimate contact between activated carbon pores and microorganisms by providing suitable attachment sites for the latter (Imai et al., 1995). Speitel and DiGiano (1987) stated that the diffusive transport was slower and the opportunities for exchange between the sorbed and liquid phases were diminished in the case of GAC particles that are much larger than PAC particles. The results of Speitel and DiGiano (1987) showed that the diffusive transport resistance in a GAC filter was the rate-limiting step in bioregeneration.

2.3.4.4. Porosity of Activated Carbon. Some researchers (Klimenko et al., 2002) stated that the porous structure of activated carbon is a factor that determines to a great extent both the rate and degree of bioregeneration. Klimenko et al. (2003) suggested that the increased mesoporosity substantially improved the access to adsorbate for bacterial degraders. In that study, a mesoporous activated carbon was found to be more bioregenerable than a microporus activated carbon, particularly at the dynamic conditions of a GAC filter. Putz et al. (2005) also suggested that most of the toluene remaining on activated carbon at the end of a bioregeneration process was likely to reside in the micropores.

2.3.4.5. Carbon Activation Type. The maximal extent of bioregeneration is dependent on the carbon activation type. Bioregeneration is considerably higher for chemically activated-wood based PAC than thermally activated-peat based PAC (Jonge et al., 1996a). In the study of Jonge et al. (1996a), chemically activated PAC exhibited a higher extent of bioregeneration than the thermally activated PAC loaded with o-cresol and 3-chlorobenzoic acid, although the thermally activated one exhibited a higher adsorptive capacity for the target compounds. This showed that the activation method and/or raw material source may have more influences on PAC adsorption properties than PAC characteristics. In the long term, a PAC type with a higher extent of bioregeneration may be a better choice due to an increased carbon service time. In other studies with a thermally activated-peat based PAC loaded with landfill leachate and a pharmaceutical wastewater, no indication of bioregeneration was observed although bioregeneration was not the major item in these studies (Çeçen and Aktaş, 2001a; Aktas and Çeçen, 2001a; Çeçen and Aktaş, 2001b). Also, in another study (Yonge et al., 1985), the thermally activated PACs, produced from peat or coal, exhibited a higher extent of irreversible adsorption compared to chemically activated wood-based GACs.

The findings of the above mentioned studies (Yonge et al., 1985; Jonge et al., 1996a; Jonge et al., 1996b) revealed that the activation method is of crucial importance regarding the extent of irreversible adsorption and/or bioregeneration. The differences in surface characteristics could have an effect on the extent of irreversible adsorption (Jonge et al., 1996b). Oxidative polymerization of phenolic compounds, which results in irreversible adsorption, is probably occurring on the surface of thermally activated carbons (Jonge et al., 1996a).

2.3.4.6 Physical Surface Properties of Carbon. Labouyrie et al. (1997) found that the physical characteristics of activated carbons (porosity, raw material) do not play an important role in the development of biological activity and they proposed that microorganisms are mainly fixed on the external surface of activated carbon. In another study (Morinaga et al., 2003), the effect of bacterial adhesion on bacterial activity was studied for different types of steam activated PACs. The authors concluded that some PAC types can stimulate bacterial activity. They also suggested that surface characteristics like

BET specific surface areas, total surface acidity, oxygen functional groups, total surface basicity, surface charge, pH_{pzc}, iodine number, metal concentrations, electric resistance, free radical concentration and formate adsorption capacity were not related to the stimulation of biological activity.

2.3.4.7. Desorption Kinetics. Desorption kinetics may also have an important role on the extent of irreversibility of adsorption. A compound, which is slowly desorbed, may be regarded as irreversibly adsorbed. In other words, adsorption of a compound may be reversible, but it may be not desorbed yet (Jonge et al., 1996b). Some bioregeneration studies have found slow rates of bioregeneration for organic compounds that desorb slowly (Goeddertz et al., 1988; Speitel et al., 1989a; Jonge et al., 1996a; Jonge et al., 1996b). The rate of desorption depends on culture conditions, fluid dynamics, metabolic activity of microorganisms and the type and density of carbon particles (Abu-Salah et al.,1996).

Speitel and DiGiano (1987) reported that a peak bioregeneration rate was achieved upon establishment of microbial activity in a GAC bed because the substrate in the outer portion of GAC was readily available to microorganisms. However, bioregeneration rate declined in time because the majority of sorption sites were limited by the diffusive transport resistance in the GAC. Hence, Speitel and DiGiano (1987) suggested that as the surface diffusivity for the substrate increased, the potential for bioregeneration also increased. Jonge et al. (1996b) suggested that the pore size distribution of activated carbon affects desorption kinetics. They found that desorption from SA4 was more gradual than CA1 and consisted of two phases. A fast initial phase of desorption, during which most of desorption took place, was followed by a slow desorption phase. This dual rate mechanism suggested that PAC (SA4) contained two regions with different diffusion modes. A network of relatively wide macropores allowed rapid diffusion, and a region of narrower micropores resulted in a relatively slower diffusion. Contrary to this, CA1 had a much opener structure than SA4 with a much higher total pore volume and mesopore volume. This caused a higher accessibility and faster diffusion for CA1.

2.3.4.8 Substrate-Carbon Contact Time. In bioregeneration studies, the contact time of the activated carbon with the substrate is also important. The bioregenerable fraction of an activated carbon-sorbed compound may decrease with the increase in this contact time

(Jonge et al., 1996a). Another study (Jonge et al., 1996b) also proved that the reversibility of adsorption varied with contact time. The desorbable fraction was found to decrease with an increase in contact time between PAC and the sorbate. During offline bioregeneration, the duration of bioregeneration was also suggested as one of the parameters having the greatest impact on the extent bioregeneration since it resulted in a higher contact time (Goeddertz et al., 1988). In the study of Goeddertz et al. (1988), increasing the bioregeneration duration from 24 to 96 hours resulted in an increase of bioregeneration from about 10-60% to about 60-75% depending on the MLVSS in the reactors. In the study of Ha et al. (2000), at a sludge residence time (SRT) of 3 days in a BAC-SBR system, the bioregeneration efficiencies in the case of phenol and 2,4-dichlorophenol were 39 and 38 %, respectively. At an SRT of 8 days, these efficiencies increased to 48 and 43 %, respectively. Hutchinson and Robinson (1990a) also reported that as the bioregeneration time increased, the breakthrough performance of phenol-loaded GAC improved when the same carbon was used for the subsequent adsorption period.

In another successive (offline) bioregeneration study performed with preloaded GAC columns (Putz et al., 2005), bioregeneration increased at higher empty bed contact times (EBCT). A higher EBCT implied a lower bulk concentration in the filter which resulted in a larger driving force for desorption and subsequent biodegradation of the sorbed compound. Also other researchers stated that bioregeneration increased with an increase in contact time and/or sludge age (DeWalle and Chian, 1977; Sirotkin et al., 2003). However, in the denitrification study of Kim et al. (1997), bioregeneration rate decreased with an increase in EBCT, although the concentration gradient was higher at higher EBCT. This was attributed to the formation of a thicker biofilm on the GAC surface at lower EBCT. The authors (Kim et al., 1997) concluded that bioregeneration was not a process merely controlled by a concentration gradient, but was more significantly affected by biological factors.

2.3.4.9 Concentration Gradient and Carbon Saturation. Bioregeneration of activated carbon may also depend on the concentration of the target compound in the bulk solution and loading on activated carbon. Putz et al. (2005) have found out that a lower equilibrium concentration in a BAC column resulted in a smaller percentage of bioregeneration. The authors (Putz et al., 2005) related the concentration dependence of bioregeneration to two

phenomena. The first phenomenon is the lower loadings on GAC surface at lower bulk concentrations which means that the adsorbate compounds are adsorbed on high-energy adsorption sites, and this eventually results in high irreversible adsorption. The effect of loading on activated carbon on the extent of bioregeneration was also investigated by Caldeira et al. (1999). The authors found that as the 4-chlorophenol loading on activated carbon was increased by increasing the amount of 4-chlorophenol in batch adsorption studies prior to contact with bacterial consortium, higher bioregeneration efficiencies were obtained in batch bioregeneration flasks upon contact with microorganisms. In spite of only 21% biodegradation of 4-CP loaded on GAC when the loading was 10.8 mg 4-CP/g GAC, bioregeneration efficiencies increased to 24% at a loading of 54 mg 4-CP/g GAC, to 56% at 108 mg 4-CP/g GAC and to 75% at 216 mg 4-CP/g GAC. The findings of Caldeira et al. (1999) can also be attributed to adsorption on high-energy sites at lower loadings.

The second phenomenon is that lower bulk concentrations have a lower potential for establishing large concentration gradients within the GAC (Putz et al., 2005). Putz et al. (2005) suggested that the process tended to be diffusion limited beyond the initial period of bioregeneration, so the smaller concentration gradients associated with lower concentrations yielded slower diffusion and thus slower bioregeneration rates. Speitel and DiGiano (1987) also reported that the liquid-phase concentration surrounding the GAC was extremely important on the rate and extent of bioregeneration during simultaneous bioregeneration. However, the authors suggested that a lower bulk concentration resulted in a higher total bioregeneration (Speitel and DiGiano, 1987). Actually, the findings of Putz et al. (2005) and Speitel et al. (1987) do not contradict each other, because the former deals with the rate of bioregeneration at the later periods of the process whereas the latter deals with the extent of total bioregeneration.

In literature it was also claimed that bioregeneration might vary temporally and spatially within a GAC column (Putz et al., 2005). A GAC column contains three zones of varying length: exhausted GAC, partially exhausted GAC (the mass transfer zone) and virgin GAC. The authors (Putz et al., 2005) suggested that the biological activity in the exhausted GAC zone held by far the most promise for bioregeneration since this exhausted zone had the most potential for desorption and subsequent biodegradation of adsorbed biodegradable organic matter.

Klimenko et al. (2003) suggested that the GAC loaded in a dynamic GAC filter was more bioregenerable compared to a GAC saturated until equilibrium at the static conditions of a batch vessel. Sirotkin et al. (2002) also suggested that the simultaneous bioregeneration occurring in a BAC filter could lead to a higher efficiency compared to successive (offline) bioregeneration of a preloaded GAC filter. However, Kolb and Wilderer (1997) reported that an offline BAC bioregeneration system led to higher degradation rates compared to a simultaneous BAC operation.

2.3.4.10. Biomass Concentration. Another determinant for the degree and rate of bioregeneration may be the concentration of biomass used in bioregeneration (Goeddertz et al., 1988). In the study of Goeddertz et al. (1988), increasing the average MLVSS concentration from 126 mg/L to 963 mg/L resulted in an increase of bioregeneration from about 10% to about 60% at the end of 24 hours. DeWalle and Chian (1977) suggested that bioregeneration was dependent on the ratio of biological solids to activated carbon solids since more carbon particles are expected to be surrounded by bacteria at high MLSS. But, Vinitnantharat et al. (2001) stated that increasing the initial MLVSS in batch bioregeneration shortened the time required to reach equilibrium, but had little effect on the magnitude of bioregeneration.

2.3.4.11. Dissolved Oxygen Concentration. Considering that the bioregeneration process includes biodegradation, the availability of dissolved oxygen (DO) in aerobic systems is also an important factor determining the degree of bioregeneration. In a literature study on the bioregeneration of a GAC column loaded with phenol (Chudyk and Snoeyink, 1984), GAC was successfully bioregenerated at an initial saturated DO concentration of 9 mg/L. However, no bioregeneration occurred when the influent DO was 4 mg/L, which was sufficient only for biodegradation of the phenol in the influent, but not for biodegradation of the loaded phenol. Putz et al. (2005) also stated that the dissolved oxygen level in GAC columns requires attention during a bioregeneration process, because in such systems a large amount of adsorbed substrate becomes very rapidly available to microorganisms.

2.3.4.12. Microorganism Type. Another important determinant for bioregeneration is the nature of the microbial population used in the bioregeneration process. In particular, in the case of slowly biodegradable compounds or organics that are classified as

nonbiodegradable in conventional biological treatment works, specific microorganisms or acclimated microorganisms are required for target compounds. For example, in the study of Jones et al. (1998), GAC loaded with the hardly biodegradable pesticide atrazine, was inoculated with an atrazine degrading bacterium *Rhodococcus rhodochrous*. The bacteria attached to GAC significantly extended the bed-life through bioregeneration of activated carbon. In the study of Caldeira et al. (1999), a bacterial consortium was isolated from the rizosphere of *Phragmitis communis* in order to develop a BAC and biodegrade the hardly biodegradable 4-chlorophenol loaded on activated carbon.

2.3.4.13. Substrate and Biomass Associated Products of Biodegradation. Ivancev-Tumbas et al. (1998) stated that the quality of the BAC surface also deteriorates during bioregeneration. The cause was either the adsorption of the lysed cells, slowly biodegradable substances and metabolites, or their reactions taking place in the system. The same authors concluded that bioregeneration can hardly be a way of avoiding deterioriation of the adsorbent quality, but can prolong the usage time. Sirotkin et al. (2002) also suggested that bioregeneration could not be an independent process for the complete recovery of the adsorption capacity of activated carbon. Marquez and Costa (1996) stated that inert biomass formed a gelatinous structure on the carbon surface and hence at high biomass concentrations (>2500 mg/L) in a PACT process, the carbon particles became trapped within the floc matrix and the carbon pores were closed. The difficulty of desorption of microbial products and filling of pores with decay products of microbial cells were also mentioned in other studies (Roy et al., 1999; Sirotkin et al., 2001; Sirotkin et al., 2002; Martin et al., 2004).

In the study of Hutchinson and Robinson (1990b), it was found that the GAC bioregenerated using recirculated microorganism-free filtered permeate performed much better than the unfiltered microbial culture recirculated through the GAC column because of microbial fouling of the column during bioregeneration in the latter case. However, in the same study, the breakthrough performance of the GAC column deteriorated gradually after each successive bioregeneration step even in the case of recirculating filtered permeate through the GAC column and a separate bioreactor. Although the authors attributed this finding to accumulation of adsorbates, irreversible adsorption of metabolic end-products might have also contributed to tis phenomenon. In the study of Nakano et al.

(2000), it was found that 82.5% of TCE adsorbed on activated carbon was bioregenerated in a successive treatment. But the TCE adsorption capacity of bioregenerated activated carbon was very low compared to virgin GAC, probably due to adsorption of extracellular metabolites from bacteria. Schultz and Keinath (1984) observed that nearly 50% of soluble microbial products (SMP) were adsorbed onto PAC in a PACT system, but only 4% of the adsorbed SMP was biodegraded by microorganisms. In the study of Zhao et al. (1999), in a BAC fluidized bed reactor that treats toluene-contaminated water, the loss of adsorption capacity was attributed to the irreversible adsorption of SMP rather than to the adsorption of toluene that is highly reversible.

2.3.5. Determination of Bioregeneration

2.3.5.1. Investigation of the Extent of Reversible Adsorption and Bioregeneration. The extent of bioregeneration is normally uncertain in such systems like PAC added activated sludge and/or BAC. Studies which report an increase in bed life in BAC systems or an increase in the removal efficiency in PACT systems do not only refer to bioregeneration, but their results also include biodegradation in these simultaneous treatment systems. However, in offline systems, where pre-loaded activated carbon is consecutively biologically treated, it is much easier to determine the extent of bioregeneration. Analysis of batch processes can provide useful design data from which continuous processes can be developed. A qualitative and quantitative study of bioregeneration is important to optimize the treatment of organic pollutants in these systems (Vinitnantharat et al., 2001). However, not many quantitative measurements of bioregeneration have been described in the literature. It is difficult to measure bioregeneration experimentally, since bioregeneration, biodegradation and adsorption occur simultaneously within these systems (Kim et al., 1997). Also, the knowledge about the extent of reversible and irreversible adsorption is a key factor for the selection of both optimal activated carbon type and refreshment rate (Jonge et al., 1996b).

Ha and Vinitnantharat et al. (2000) expressed the reversibility of adsorption as a degree of hysteresis (w). The fact that the 1/n value in the desorption isotherm is slower than the 1/n value in the adsorption isotherm implies the intensity of desorption to be slower than the intensity of adsorption. Their results showed that high GAC dosages

resulted in a high degree of hysteresis (irreversibility). Therefore, the authors suggested that low GAC dosages should be applied to achieve a high reversibility of adsorption in the BAC system.

In the study of Jonge et al. (1996a), it was found that the bioregenerable fraction did not exceed the leachable fraction. This showed that the bacteria were unable to reach non-desorbable compounds or to influence the desorbability of the compounds. These results proved that bioregeneration is controlled by the desorbability of the compounds, but did not favor the bioregeneration theory of desorption of organic matter according to exoenzymatic reactions. Regarding this, the authors suggested that the term bioregeneration should be used with caution. Regeneration of loaded activated carbon could also have been achieved through abiotic desorption, e.g. by leaching of the loaded activated carbon. Some authors prefer to use the term only for cases when a direct interaction has been shown between the microorganisms and the adsorbed compound (Xiaojian *et al*, 1991). However, most authors use the term bioregeneration as long as microorganisms are responsible for the removal of the dissolved compound in the bulk fluid, leading to desorption of the sorbed compound (Schultz and Keinath., 1984, Speitel et al., 1989a).

2.3.5.2. Investigation by Scanning Electron Microscopy. Scanning electron microscope (SEM) analyses help to determine if the microorganisms attach on the outer surface of carbon or inside the carbon. Regarding that microorganisms tend to attach near substrate for an efficient uptake, it can be expected that microorganisms attached to inner side can significantly contribute to the assimilation of adsorbed compounds. This microbial activity enhances the bioregeneration of activated carbon by inducing a progressive desorption of adsorbate to the bulk solution (Ha et al., 2000) or by excreting extracellular enzymes through the activated carbon pores. In the study of Vuoriranta and Remo (1994), SEM analyses showed that bacteria (filamentous and rod-shaped) were present only inside the holes and pores, but not on the surfaces of GAC particles in a fluidized bed reactor with a turbulent fluid dynamics. In another study (Abu-Salah et al., 1996), SEM micrographs showed that bacteria were attached on both the interval cavities and outer surface of PAC particles. In another bioregeneration study with azo dyes, SEM investigations have shown

that bacteria successfully colonized the macropore structure of GAC (Walker and Weatherley, 1998).

In the study of Ha et al. (2000), SEM analysis also showed that at high sludge residence times (SRT), the micropores in the activated carbon were more densely covered with microorganisms. This higher attachment of microorganisms probably increased the bioregeneration efficiency through assimilation of easily accessible organic compounds adsorbed on activated carbon.

2.3.5.3. Investigation of Bioregeneration in Multiple Substrate Systems. Most of the research on bioregeneration has focused on a single component only. However, wastewaters usually contain multiple components that might influence desorption of compounds and biodegradation. Putz et al. (2005) stated that mixtures of organic compounds are difficult to treat because each compound might vary in its ability to be biodegraded or adsorbed. Bioregeneration of activated carbon was studied in the literature for two competing compounds (phenol and 2,4 dichlorophenol) simultaneously present in the BAC system (Ha and Vinitnantharat, 2000; Ha et al., 2000). In such systems, the percentage of bioregeneration of adsorbate is related to the biodegradability and degree of hysteresis for each compound (Ha and Vinitnantharat, 2000). In that study, the bioregeneration of 2,4-DCP in a bisolute system was found to be higher than in a single solute system, probably due to the cometabolic removal of the less biodegradable compound. Bioregeneration of phenol was suppressed in the bisolute system, probably due to competition for adsorption sites. Pannu et al. (2003) stated that the presence of peanut oil accelerated the biodegradation of polycyclic aromatic compounds (PAHs) and this resulted in partial bioregeneration of activated carbon loaded with anthracene.

Putz et al. (2005) investigated the bioregeneration in GAC columns using mixtures of biodegradable (benzene or toluene) and nonbiodegradable (perchloroethylene or carbon tetrachloride) synthetic organic chemicals (SOC). Pre- and post-experimental GAC loadings showed a marked decrease in the biodegradable SOC loading as well as an increase in the nonbiodegradable SOC loading. Bioregeneration of GAC occurred in the case of the biodegradable SOC and the regenerated pores of GAC were loaded with the nonbiodegradable SOC in that study (Putz et al., 2005). Also in another study (Erlanson et

al., 1997), when the biodegradable and nonbiodegradable organics had similar adsorbabilities, significant increases in the GAC service life of up to 1.5 times were predicted compared to adsorption alone with respect to the nonbiodegradable compound. In the study of Putz et al. (2005), when the biodegradable organic compound was weakly adsorbed on activated carbon, only a small increase in the adsorption capacity was observed for the nonbiodegradable compound because weakly adsorbed chemicals do not compete as well for GAC adsorption sites, so their biodegradation does not reopen as many adsorption sites. Bioregeneration increased with the adsorbability of the biodegradable compound in binary systems consisting of a biodegradable and a nonbiodegradable compound (Putz *et al*, 2005).

2.3.5.4. Bioregeneration of Adsorbents other than Activated Carbon. In literature, bioregeneration was also determined for adsorbent materials other than activated carbon (Lee and Lim, 2003; Syamsiah and Hadi, 2004). Lee and Lim (2003) showed that the phenol and alkyl phenol adsorption capacity of rice husk was increased in a biological sequencing batch reactor indicating bioregeneration of the rice husk. Desorption of phenol and alkyl phenols from the activated rice husk took place at a faster rate than from powdered activated carbon, although the adsorption capacity of PAC was much higher than the rice husk.

In the study of Syamsiah and Hadi (2004), bioregeneration was determined for phenol-loaded natural zeolite. In that study, about two per cent of the adsorbed phenol was microbially degraded into carbon dioxide, whereas the rest could be converted into dissolved products and microbial mass and/or irreversibly adsorbed. Silva et al. (2004) found out that the resin Amberlite XAD-4, a synthetic resin constituted by a non-ionic crosslinked polymer, loaded with a herbicide named molinate was successfully bioregenerated, whereas the activated carbon used in their study was not bioregenerated, because molinate desorbed from the resin, but not from activated carbon.

Also in the study of Walker and Weatherley (1998), the activated bone char loaded with azo dye was successfully bioregenerated by a consortia of bacteria, whereas no bioregeneration was observed with activated carbon due to the poor dye desorption from activated carbon. Li et al. (1998) determined the bioregeneration of the char produced from

low rank coal briquette loaded with p-nitrophenol. Martin et al. (2002) and Martin et al. (2004) also found similar removal performances for carbonaceous adsorbents produced from surplus biological sludge and commercial activated carbon although the latter had much higher adsorption capacity. However, the authors did not attribute this finding to the reversibility of adsorption because phenol was reversibly adsorbed on both types of carbon (Martin et al., 2002; Martin et al.; 2004). The same authors suggested that bioregeneration might be impaired by the obstruction of pores due to bacterial growth in the case of commercial activated carbon that had a narrower pore size distribution compared to the mesoporous sludge-based adsorbent (Martin et al., 2004).

2.3.6. Quantification of Bioregeneration

Bioregeneration of activated carbon can be quantified either by the direct measurement of substrate loading on the activated carbon, or by the indirect measurement of substrate consumption such as CO_2 production using either a radiochemical analysis technique or instrumental measurement of respiration. Examples for quantification of bioregeneration are presented in Table 2.1.

2.3.6.1. Use of a Freundlich Adsorption Analogy. In the study of Vinitnantharat et al. (2001), bioregeneration was carried out in a batch culture containing loaded GAC, acclimated microorganisms, mineral salt solution, and adsorbate. Bioregeneration was quantified using the Freundlich adsorption isotherm constants and equilibration concentrations. GAC was initially equilibrated with a known concentration of adsorbate before bioregeneration. After saturation, the concentration in supernatant was measured to determine the amount of adsorbate adsorbed before bioregeneration. This mixture was then inoculated with acclimated microorganisms and mineral salts solution. The mixture was aerated and the residual adsorbate concentration was measured at different time intervals. At the end of bioregeneration, the supernatant was decanted and GAC was rinsed and put into an autoclave to arrest the activity of microorganisms. Consequently, the sterilized GAC was reloaded by transferring it to the reactor and equilibrating with a known concentration of adsorbate, and residual adsorbate concentration was measured.

Table 2.1. Examples of bioregeneration efficiencies and process conditions reported in literature.

Compound	Activated Carbon	Important Carbon Characteristics	Biomass	Reactor Type	Bioregen. (%)	Reference
Phenol	PAC Westvaco SA15	chemically activated	Acclimated to substrate	Batch	100	Schultz and Keinath (1984)
Phenol	GAC Calgon F-400	-	Acclimated to substrate	GAC column	17.7-75.2	Goeddertz et al. (1988)
Phenol (trace)	GAC	-	Acclimated to substrate	BAC column	8-15	Speitel and DiGiano(1987)
p-nitrophenol (trace)	GAC	-	Acclimated to substrate	BAC column	5-22	"
Phenol	GAC Sigma	lignite based	Acclimated to substrate	Batch	67.3-83.8	Ha et al. (2001)
2,4-Dichlorophenol	GAC Sigma	lignite based	Acclimated to substrate	Batch	51.7-64.3	"
Phenol	GAC Sigma	lignite based	Acclimated to substrate	Batch	74.5-76.3	Ha and Vinitnantharat(2000)
2,4-Dichlorophenol	GAC Sigma	lignite based	Acclimated to substrate	Batch	56.8-60.2	"
Phenol	GAC Sigma	lignite based	Acclimated to substrate	Batch	31.4	Vinitnantharat et al. (2001)
2,4-Dichlorophenol	GAC Sigma	lignite based	Acclimated to substrate	Batch	14.3	"
Phenol	GAC Sigma	lignite based	Acclimated to substrate	BAC-SBR	39-48	Ha et al. (2000)
2,4-Dichlorophenol	GAC Sigma	lignite based	Acclimated to substrate	BAC-SBR	38-43	"
Phenol	PAC	-	Acclimated to substrate	SBR	77±4	Lee and Lim, 2005
p-methylphenol	PAC	-	Acclimated to substrate	SBR	69±4	"
p-ethylphenol	PAC	-	Acclimated to substrate	SBR	68±4	"
p-isopropylphenol	PAC	-	Acclimated to substrate	SBR	58±6	"
4-Chlorophenol	GAC Sigma	-	*Phragmitis communis*	Batch	21-75	Caldeira et al., 1999
o-cresol	PAC Norit SA4	thermally activated	activated sludge	Batch	15	Jonge et al. (1996a)
3-chlorobenzoic acid	PAC Norit CA1	chemically activated	*Pseudomonas B13*	Batch	85	
Tetrachloroethylene	GAC Calgon F-400	-	*Rhodococcus rhodochrous*	BAC column	39.4	Putz et al., 2005
Toluene	GAC Calgon F-400	-	*Rhodococcus rhodochrous*	BAC column	45.5	"
Benzene	GAC Calgon F-400	-	*Rhodococcus rhodochrous*	BAC column	38.2	"
Carbontetrachloride	GAC Calgon F-400	-	*Rhodococcus rhodochrous*	BAC column	28.8-33.2	"
Trichloroethylene	GAC Calgon F-400		Phenol-utilizing bacteria	GAC column	82.5	Nakano et al., 2000
Surfactants mixture	GAC AG-3	microporous	*Pseudomonas sp.*	Batch	12-35	Kilmenko et al., 2003
Surfactants mixture	GAC AG-PR	-	*Pseudomonas sp.*	Batch	11-29	"
Surfactants mixture	GAC SKNP-1	mesoporous	*Pseudomonas sp.*	Batch	8-23	"
Surfactants mixture	GAC AG-3	microporous	*Pseudomonas sp.*	GAC column	59	"
Sulfonol	GAC AG-3	microporous	*Pseudomonas sp.*	GAC column	22	"
Surfactants mixture	GAC SKNP-1	mesoporous	*Pseudomonas sp.*	GAC column	69	"
Non-iogenic surfactants	GAC SKT-3	-	Acclimated to substrate	BAC filter	20-24	Sirotkin et al., 2001
Polyoxyethylen	GAC SKT-3	microporous	Acclimated to substrate	GAC filter	50-53	Sirotkin et al., 2002

2.3.6.2. Direct Measurement by Using Adsorption Capacities. Bioregeneration was quantified in the study of Klimenko et al. (2003) by the proportion between the equilibrium adsorption capacities of fresh and bioregenerated activated carbons as seen in Equation 2.7 below.

$$\% \text{ bioregeneration} = 100 * \alpha_{reg.}/\alpha \quad (2.7)$$

where $\alpha_{reg.}$ and α stand for equilibrium adsorption capacities of regenerated and fresh activated carbon, respectively. Goeddertz et al. (1988) used a similar calculation method for a GAC column. Hutchinson and Robinson (1990 a,b) determined the extent of bioregeneration using breakthrough curves for both fresh and bioregenerated GAC columns.

2.3.6.3. Direct Measurement by Solvent Extraction. In the study of Ha and Vinitnantharat (2000), the bioregeneration of GAC loaded with a single solute or bisolute was studied in batch reactors using a direct measurement method. GAC was initially equilibrated with a known concentration of adsorbate before bioregeneration. After saturation, the supernatant was taken out and the concentration in the supernatant was measured. The GAC was then contacted with a known weight of acclimated microorganisms. The amount of adsorbate remaining on GAC was monitored over a time period. The mass of compound remaining on activated carbon was extracted with methylene chloride by a Soxhlet extractor before measurement. The percentage of bioregeneration was calculated by the following equation:

$$\% \text{ bioregeneration} = 100 * (Q_1-Q_2)/Q_1 \quad (2.8)$$

where

Q_1 = the initial amount of adsorbate adsorbed on GAC, g

Q_2 = the amount of adsorbate remained after contact with biomass, g

Putz et al. (2005) also used a direct measurement method to determine the loading on GAC columns by extraction with a proper solvent, methanol. In that study, small samples of GAC were taken before and after each bioregeneration experiment, and placed into a headspace-free vial containing methanol and mixed for four days.

2.3.6.4. Quantification during Simultaneous Adsorption-Biodegradation. Unlike previously mentioned researchers, Walker and Weatherley (1999) quantified bioregeneration in batch systems with simultaneous adsorption and biodegradation. For this purpose, they used four batch systems with a) bacteria immobilized on GAC, b) bacteria immobilized on sand, c) GAC with no biological activity, and d) free bacterial cells. They used the bacterial cells immobilized on sand to investigate the advantage of immobilization over free cells in suspension. Higher biomass growth rates were suggested for immobilized cells because of the higher substrate concentration on the activated carbon surface than in the bulk solution. The immobilization of cells on activated carbon can also increase the degradation of the target compound due to a decrease in toxic substances in the cell environment. The researchers (Walker and Weatherley,1999) used nonbiodegradable azo dyes and biodegradable anthraquinone dyes as adsorbates. They determined the improvement in removal as the difference between the removal obtained by cells immobilized on GAC (BAC system) and the total removal obtained by cells immobilized on sand and by GAC with no biological activity. In most real cases, this difference may include both bioregeneration and stimulation of biological activity by adsorption of toxic substances onto activated carbon. However, if the compound used in the bioregeneration study is not toxic to microorganisms, then this difference can be considered to be the result of bioregeneration only. In another study, the same authors used their approach in BAC modelling with acid dyes using the Monod equation to describe biodegradation kinetics (Walker and Weatherley, 1997). They found that no desorption and therefore no bioregeneration took place from BAC in the case of acid dyes.

2.3.6.5. Use of Measurement of Biodegradation Products. In the study of Jonge et al. (1996a), bioregeneration was determined by measuring CO_2 production in a batch culture. Determination of CO_2 was accurate and fast, allowing a time evaluation of the status of the experiments. The batch culture used in bioregeneration studies contained acclimated biomass, a mineral salts medium and a known amount of centrifuged, preloaded PAC. The PAC saturated with the adsorbate was separated by centrifugation and the PAC pellet obtained after centrifugation was used for bioregeneration experiments. Batches with the following compositions were used as blanks: unloaded PAC with biomass, biomass without PAC and loaded PAC without biomass. At the end of the experiments, when the CO_2 curves had reached a stable level indicating that growth had ceased, all batches were

acidified to force all CO_2 into the headspace. The percentages of CO_2 in the headspaces were determined using a gas chromatograph. The authors state that the direct measurement of bioregeneration was unsuitable due to the contact time dependent recoveries during the extractions of loaded PAC.

Another method for quantitative determination of bioregeneration was using radiolabelled compound with ^{14}C. In the effluent stream, the amount of CO_2 containing ^{14}C and the radiolabelled substrate showed that pre-adsorbed compounds had been desorbed, thus the amount of bioregeneration could be calculated (Schultz and Keinath, 1984; Speitel and Digiano., 1987; Putz et al., 2005).

Another indirect way of determining biodegradation of organic matter adsorbed on activated carbon is the measurement of the chloride ion in the case of chlorinated organic compounds such as trichloroethylene in the study of Nakano et al. (2000) and 4-chlorophenol in the study of Caldeira et al. (1999).

In anaerobic biological systems combined with activated carbon, bioregeneration was determined and/or quantified by measurement of biogas productions (Suidan et al., 1980; Kim et al., 1986). In the study of Sa and Malina (1992), bioregeneration was determined using radiolabelled phenol in the feed and by the measurement of radiolabelled methane and carbon dioxide in the gaseous effluent of an anaerobic GAC biofilm.

2.3.6.6. Use of Respirometry in Aerobic Systems. The potential applicability of respirometric methods for monitoring bioregeneration of activated carbon was also investigated in the literature (Ivancev-Tumbas et al., 1998). The activity of microorganisms present on carbon was estimated using the Warburg's apparatus in that study. The specific rate of oxygen consumption was monitored during bioregeneration. For this purpose, an amount of activated carbon was taken from the column several times during bioregeneration, then placed in a bottle with dilution water for BOD_5 and KOH solution, and the oxygen consumption was monitored for 5 to 7 days. The authors also determined bioregeneration by a direct method through the measurement of adsorbate remaining on activated carbon.

Lee and Lim (2005) also quantified bioregeneration by oxygen uptake measurements with a manometric respirometer placed into a constant temperature cabinet. The authors compared the oxygen consumption in biodegradation only with those in the simultaneous adsorption and biodegradation of phenol and alkyl-substituted phenols. The substrate removal by biodegradation was determined by oxygen uptake, and subtracting this amount from the initial substrate concentration yielded the amount of substrate adsorbed by activated carbon. The difference between the initial and final loading on carbon equaled the amount bioregenerated.

2.3.6.7. The Kinetics of Bioregeneration. Vinitnantharat et al. (2001) also calculated a bioregeneration rate constant assuming that the microbial activity followed first-order kinetics. The first-order pattern was also used in other literature studies to define the bioregeneration kinetics with respect to adsorbed quantity (Ivancev-Tumbas et al., 1998; Kim et al., 1997). The rate of bioregeneration at any time (t) was proportional to the amount of adsorbate left on GAC at that particular time. Hence, the renewal of active sites occurred very fast at the beginning and declined gradually.

In the study of Ivancev-Tumbas et al. (1998), the rate of bioregeneration was found to be dependent on the substrate. Bioregeneration was slower when the substrate was a mixture of substituted phenols than phenol alone. In the study of Vinitnantharat et al. (2001), the first-order bioregeneration rate was found to be lower for 2,4-dichlorophenol compared to that of phenol.

2.3.7. Mathematical Modelling Approaches

Hutchinson and Robinson (1990a) suggested that a bioregeneration model required the mathematical description of two distinct processes: the kinetics of adsorption/desorption and the kinetics of microbial growth and solute degradation. Several models have been developed in literature, which incorporated various simplifying assumptions for mass transport resistance, microbial kinetics and biofilm thickness in order to gain an understanding of the biological activated carbon (BAC) process (Speitel et al., 1987). Speitel et al. (1987) developed and tested a more elaborate model that accounted for bioregeneration. They used this model to predict the sorbed amount as well as the bulk

liquid concentration. The model considered a single substrate, phenol or *p*-nitrophenol at trace concentrations. The model described adsorption by the Freundlich isotherm and surface diffusion in the adsorbent, and described biodegradation by Monod kinetics and diffusion through the biofilm on the GAC. The model also included the film transport resistance, the growth and decay of the biofilm over time, but excluded the transport resistance at the GAC/biofilm interface. The model performance was compared with the experimental data obtained by measurements of radiolabelled phenol or *p*-nitrophenol and CO_2. The model was initialized after an acclimation period during which sufficient biomass attachment was achieved and after the achievement of the maximum bioregeneration rate. The model was able to adequately predict bioregeneration rates when the biomass loss coefficient was increased above its calculated value. However, the predicted liquid phase concentrations were higher than the measured values. The authors concluded that the model was not largely predictive because of an inadequate understanding of biofilms, including density, diffusional transport resistance and loss rate through shearing at the time of the study.

Goeddertz et al. (1988) presented a predictive model in an offline GAC bioregeneration system using phenol as the model compound. The Freundlich adsorption isotherm constants, the external mass transfer through the biofilm and the Haldane type biodegradation kinetics were incorporated into the model. The model successfully predicted the bulk liquid substrate concentrations as well as the extent of bioregeneration. The authors stated that significant bioregeneration occurred when desorption was the limiting reaction rather than biodegradation as shown with experimental data and the predicted model.

A model was developed by Hutchinson and Robinson (1990a) to predict a bioregeneration process for single (phenol) and bi-solute (phenol and *p*-cresol) systems in a fixed-bed GAC adsorber. The model included intraparticle mass transfer, film mass transfer, description of adsorption equilibrium by the multi-solute Fritz-Schluender isotherm equation, assumption of plug flow in GAC column, assumption of CSTR conditions for the separate bioregeneration reactor, description of microbial kinetics by Haldane kinetics for single substrate and by a simplified growth model that assumed a constant specific growth rate for the two components in the bi-solute system. The authors

suggested that the model could be used to predict the breakthrough behavior of both fresh and bioregenerated activated carbon. However, in multi-solute systems, the model was only applicable when the substrates were similar in terms of growth parameters, such as phenol and *p*-cresol. In another study, this model could not predict the breakthrough of a bioregenerated GAC (Hutchinson and Robinson, 1990b) in the case of a microbial culture was recirculated through the GAC column. The reason for this was microbial fouling of carbon pores, which significantly deteriorated the carbon capacity. But in the same study, the model agreed well with the findings in GAC bioregeneration using recirculated microorganism-free filtered permeate through the GAC column. In that case the presence of a separate bioreactor had prevented microbial fouling. In the bi-solute system, the model tended to overpredict effluent phenol concentrations and slightly underpredict effluent *p*-cresol levels. (Hutchinson and Robinson, 1990b).

Mathematical models have been also developed to predict the bioregeneration in BAC columns treating mixtures of biodegradable and nonbiodegradable organic compounds (Putz et al., 2005). Erlanson et al. (1997) described a two-component equilibrium-based model called the biodegradation/adsorption–screening model (BASM). Putz et al. (2005) suggested that modelling becomes much simpler by considering only equilibrium situations in BAC columns compared to that of kinetic models. The BASM model helped to determine which adsorption-only situations could possibly benefit from employing simultaneous biodegradation and adsorption by applying several hundreds of hypothetical scenarios. Speitel et al. (1989b) developed a kinetic model called the multiple component biofilm diffusion biodegradation and adsorption model (MDBA) that described both adsorption and biodegradation in multicomponent GAC columns. The MDBA model combined the single-component adsorption and biodegradation model developed by Speitel et al. (1987) with the ideal adsorbed solution theory (IAST). The MDBA model adjusted the IAST equation by using a correction factor to account for differences between predicted and measured equilibrium concentrations, assumed a homogeneous surface diffusion considering that pore diffusion was insignificant, and assumed that biodegradation of multicomponents occurred simultaneously in order to simplify the model. In the study of Putz et al. (2005), a good correlation was found between the MDBA model fits and the measured effluent concentrations and between the simulated and measured loadings on activated carbon. The authors (Putz et al., 2005) used the model to

predict the cumulative bioregeneration over time. In that study, the measured bioregeneration occurred more rapidly than the simulated model, but the same value was reached at the end.

The method for quantification of bioregeneration using the analogy of the Freundlich adsorption concept (Vinitnantharat et al., 2001), as described in the previous section, was used in another study to develop a predictive isotherm model to evaluate the extent of bioregeneration (Ha et al., 2001). In that study, the modelling of bioregeneration was conducted with phenol and 2,4-dichlorophenol in both single- and bi-solute systems and also in the presence and absence of biodegradation by-products. The loadings on activated carbon in batch and BAC sequencing batch reactors (BAC-SBR) were estimated using the Freundlich constants as described in the previous section. The results were compared with the experimentally obtained loadings using the direct measurement method that employed extraction with methylene chloride. When metabolic by-products were not included in the isotherms, the loadings on carbon were overestimated. But when metabolic by-products were included in the isotherms, the model predicted bioregeneration with reasonable consistency in the case of both single and bi-solute systems and in both batch and BAC-SBR reactors. Ha et al. (2001) showed that in the modelling of bioregeneration, metabolic intermediates and by-products should be taken into account since they decrease the adsorption capacity of activated carbon.

2.3.8. Overall Discussion on the Literature Work

Some authors preferred to use the term “bioregeneration” as long as a direct reaction between microorganisms and the adsorbed compound has been demonstrated. But most of the authors used the term bioregeneration when the adsorptive capacity of activated carbon was renewed through the action of microorganisms. As long as the adsorbate/substrate is removed from the bulk liquid by microorganisms, the process should be called “bioregeneration”. Continuous biodegradation of the adsorbate/substrate in the bulk liquid leads to a continuous concentration gradient between the carbon and bulk liquid. The consequence is the continuous desorption from activated carbon until equilibrium. Therefore, it should be emphasized that desorption serves as a prerequisite step for bioregeneration in any case.

There are also literature studies showing that bioregeneration may also be caused by the activity of exo-enzymes. Exo-enzymatic reactions inside the carbon pores resulted in higher bioregeneration rates than would be expected from desorption only due to concentration gradient. However, the effect of exo-enzymatic reactions on bioregeneration is still a hypothesis because it was not evidenced by a direct measurement of the enzyme reactions inside the pores. The characteristics of the microbial community and/or the nature of the microbial ecosystem seem to be important factors for exo-enzymatic reactions.

Bioregeneration is dependent on several factors including the biodegradability, adsorbability and desorbability of the sorbate, the characteristics of activated carbon and the process configuration. Literature studies have shown that the bioregeneration process can increase the service-life of activated carbon and can be optimized by flexible operation measures. Future research is still required to determine the optimum conditions for an increased bioregeneration. In particular, activated carbon type, nature of the microbial community, the optimum process configuration and operational conditions need further investigations.

Literature studies have shown that activated carbons, which adsorb more reversibly, should be preferred for an optimum bioregeneration. Depending on the characteristics of the wastewater, the most bioregenerable activated carbon can be determined in each case. The nature of the microbial ecosystem is also very important in terms of bioregeneration. Many studies in literature used specialized microorganisms or microorganisms acclimated to the wastewater. Molecular tools can be used in future work to determine which type of microorganisms result in better bioregeneration performances. The outcome of several studies showed that process configuration is particularly important in terms of bioregeneration. Offline bioregeneration systems seem to be applicable for regeneration of activated carbon loaded in conventional adsorption processes. Bioregeneration in simultaneous biodegradation and adsorption systems can also be optimized by changing the process configuration, e.g. by applying variable organic loadings to the system.

3.MATERIALS AND METHODS

3.1. Activated Carbon Characteristics

Norit SA4 and its granular countertype PKDA are peat based carbons, which are steam activated at 850-1000°C. Norit CA1 and its granular countertype CAgran are wood based carbons produced by chemical activation using the phosphoric acid process at a temperature of 500°C. After the activation process, the bulk of the acid is recovered through water washing. Both carbons (CA1 and CAgran) have a very open (macro/meso) pore structure which results in a high adsorption capacity for higher molecular weight organics and an effective regeneration. The characteristics of the four commercial carbon types can be seen in Table 3.1.

Table 3.1. Characteristics of activated carbons used in the experiments.

Activated Carbon	**SA4**	**CA1**	**PKDA**	**CAgran**
Physical form	powdered	powdered	granular	granular
Activation method	thermal	chemical	thermal	chemical
Apparent density (g/L)	545	370	295	225
Moisture (%)	2	11	2	12
Ash content (%)	8	2	8	3
Molasses Number	525	180	-	165
Methylene Blue adsorption (g/100g)	11	29	-	29
Iodine number (mg/g)	700	-	750	
Total Surface Area (BET) (m^2 /g)	800	1400	850	1400
Particle size distribution	D_{10}=3μm	D_{10}=7μm	0.5%>2 mm	1%>1.7 mm
	D_{50}=29μm	D_{50}=28μm	99%>0.59mm	2%<0.84mm
	D_{90}=161μm	D_{90}=75μm		
Porosity				
Total pore volume (cm^3 /g)	0.80	1.55	1.19	1.64
Micro (Φ<2nm)	0.25	0.45	0.30	0.40
Meso(Φ 2-50nm)	0.19	0.75	0.19	0.70
Macro(Φ >50nm)	0.36	0.35	0.70	0.54

All activated carbon samples were obtained from the Norit Company, the Netherlands. The surface areas of the carbons were determined by the Norit Company from the N_2 isotherm according to BET 3 parameters method. Following the IUPAC recommendations, the pore size distribution was determined by the Norit Company according to the volume distribution method which was based on analytical data consisting of the bulk density (ASTM), the density in mercury, mercury porosimetry, density in benzene and benzene adsorption isotherm. The macropore volume was taken from the standard listing of porosimetry results as the difference between the volumes at Φ = 50 nm and Φ = 15000 nm. The mesopore and micropore volumes were calculated by the Norit Company from the benzene isotherms using the Kelvin method. The micro- and mesopore volumes, dominating the adsorptive capacities, do not differ very much between the powdered and granular countertypes. However, in the production of powdered carbons, the larger macropores are milled away, resulting in significant differences compared to the granules.

3.2. Characteristics of the Target Phenolic Compounds

The main characteristics of the target phenolic compounds can be seen in Table 3.2. Figure 3.1 illustrates the molecular shape of these compounds.

Table 3.2. Properties of the model phenolic compounds used in the experiments.

	Phenol	**2-Chlorophenol**	**2-Nitrophenol**
Molecular Formula	C_6H_6O	C_6H_5ClO	$C_6H_5NO_3$
Molecular weight (g/mol)	94.11	128.56	139.11
Melting Point (°C)	43	9.3	43-45
Boiling Point (°C)	181.7	174.9	215
Density (g/cm^3) at 20/4 °C	1.06	1.26	1.49
Dissociation constant at 25°C	9.99	8.48	7.23
Solubility in water (mg/L) at 25°C	82000	28000	2100
Vapor pressure (10^{-2} mm Hg) at 25°C	62	1.42	11.25
Octanol/Water partition Coefficient (log K_{ow})	1.37-1.75	2.15-2.25	1.79

Phenol 2-Chlorophenol 2-Nitrophenol

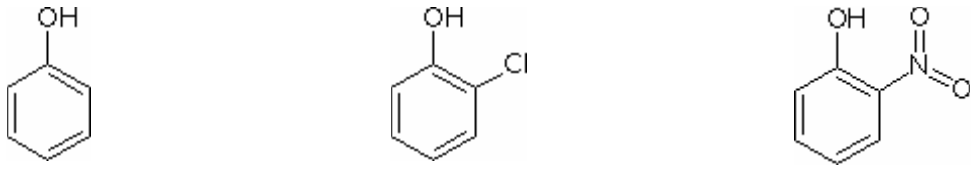


Figure 3.1. Molecular structures of phenolic compounds used in experiments.

Phenol is used in the production of epoxy and phenolic resins, pharmaceuticals, germicides, fungicides, herbicides, dyes and acids, and as a disinfectant and antiseptic. Exposure to phenol may cause liver and kidney damage. It may also cause adverse reproductive and mutagenic effects. Repeated skin contact may cause dermatitis with dark pigmentation of the skin. It may also cause severe and permanent damage to the digestive tract and nervous system. Inhalation of phenol causes severe irritation of upper respiratory tract.

2-Chlorophenol is an intermediate in chemical production of fungicides, bactericides, antiseptics, disinfectants, wood and glue preservatives, and can also be produced in the chlorination of drinking water and sewage. It exerts toxicity through skin absorption, inhalation and ingestion. Its ingestion causes decrease in respiration and blood pressure, weakness and death at high doses. Exposure to 2-chlorophenol may cause lung, liver and kidney damage. Repeated skin contact may cause dermatitis. It may also cause adverse effects on the nervous system and severe depression.

2-Nitrophenol is used in the production of dyes, photochemicals, pesticides, wood preservatives, explosives and leather treatments. Inhalation of 2-nitrophenol causes irritation to the respiratory tract and can be a route of absorption by the body. It is toxic if swallowed and can cause formation of methaemoglobin. Exposure to high concentrations may cause breathing trouble, fall in blood pressure, convulsions and death. Contact with skin and eyes causes irritation and pain. Chronic exposure may damage the liver and kidneys.

3.3. Experiments with Phenol

3.3.1. Adsorption Experiments with Phenol

The first step in the experimental work consisted of the determination of phenol adsorbability for each activated carbon type in batch adsorption tests. Adsorption studies were performed in 250 mL flasks shaken at 140 RPM and 25°C. Memmert SV 1422 horizontal shaker was used in all adsorption and desorption experiments throughout the study. For determination of the adsorption isotherms, different masses of activated carbon (200-1500 mg/L) were contacted with fixed concentrations of phenol (200 mg/L) and the mixture was agitated until reaching equilibrium. The time required for reaching an equilibrium phenol concentration is defined as the equilibrium time for adsorption. For this purpose, phenol and soluble COD (SCOD) concentrations were determined with respect to time until reaching equilibrium concentrations. The equilibrium time was determined as 2 days for SA4, CA1 and CAgran, and 3 days for PKDA. Finally, initial and final equilibrium concentrations in the adsorption flasks were measured and used for the construction of adsorption isotherms. This procedure was used in all adsorption experiments throughout the study.

3.3.2. Desorption Experiments with Phenol

The second step of the experimental work consisted of the determination of phenol desorbability for each activated carbon type in batch desorption tests. Desorption studies were performed in 100 mL flasks shaken at 140 RPM and 25°C. Desorption of the target compounds was conducted to determine the degree of reversibility of adsorption for each activated carbon. Desorption isotherms were constructed according to a batch-displacement technique (Jonge et al., 1996b, Ha and Vinitnantharat, 2000; Vinitnantharat et al., 2001). A known weight of activated carbon (2000 and 4000 mg/L) was initially contacted with a known concentration of phenol and was agitated until equilibrium was reached as in adsorption batches. After adsorption equilibration, the supernatant was removed by centrifugation and the sorbate concentration in the supernatant was measured. Hettich Rotofix 32 centrifuge was used in all centrifugation works throughout the study. The supernatant was replaced by distilled water. Desorption of phenol from activated

carbon occurred until equilibrium. Desorption equilibrium for phenol was previously determined as 24 hours. Upon equilibration, the concentration in the liquid phase was measured. Desorption was conducted successively until the phenol concentration in the supernatant was under the detection limit of 0.1 mg/L. This procedure was used in all desorption experiments throughout the study.

A succession of desorption steps produced a desorption isotherm. After each successive desorption step, the new hypothetical activated carbon loading for the following desorption step (q_i) was calculated by subtracting the amount of desorbed phenol from the activated carbon loading at the beginning of the desorption step as shown by Equation 3.1.

$$q_i = \frac{X_a - \sum (X_d)_i}{M} \tag{3.1}$$

where X_a is the initial phenol loading on the carbon (mg), X_d is the mass of phenol desorbed after each i^{th} desorption step (mg) and M is the mass of activated carbon used in each desorption experiment (g). The pH values in the desorption experiments ranged between 5.6-7.2 for the thermally activated carbons, and between 4.6-6.9 for the chemically activated carbons. These pH values were below the pK_a value of 9.99 (at 25°C) for phenol.

3.3.3. Biodegradation and Bioregeneration Experiments with Phenol

Biodegradability and bioregenerability of phenol was studied in simultaneously operated batch reactors containing activated sludge. The experimental set-up (Figure 3.2) consisted of 2 L plexiglass batch reactors provided with four baffles to prevent vortex formation and thereby obtain proper mixing conditions. The reactors were placed in a hood and were surrounded by water jacket for temperature control. Conic covers closed the tops of the reactors. Spent air was removed by a hose and passed through a gas-wash bottle, which contained water or methylene chloride. Air was supplied by a compressor to provide sufficient oxygen. Mixing was obtained by magnetic stirrers instead of excessive aeration to prevent volatilization of phenol. The temperature in the reactors ranged between 22-25°C, and dissolved oxygen concentrations were always above 4 mg/L.



Figure 3.2. Simultaneously operated batch activated sludge reactors; left: biodegradation reactor without activated carbon and right: bioregeneration reactor with loaded activated carbon.

Microorganisms were obtained from a laboratory-scale batch activated sludge reactor which was semi-continuously fed with a synthetic wastewater as in other studies (Aktaş and Çeçen, 2001a; Çeçen and Aktaş, 2001b). This mother reactor was constantly fed with synthetic domestic wastewater at a daily loading rate of 500 mg COD/L/day and the sludge age was adjusted to 20 days by withdrawing each day one twentieth of the mixed liquor. The activated sludge had an MLVSS/MLSS (Mixed Liquor Volatile Suspended Solids/Mixed Liquor Suspended Solids) ratio of about 0.8. Samples of environmental scanning electron micrographs of this activated sludge are shown in Figure 3.3. Other samples can be seen Figure B.1 in Appendix B. Figure 3.3 shows the floc structure of the original activated sludge. These flocs settled very well in activated sludge reactors. Most of the microorganisms were cocci-shaped bacteria with about 1μm diameter. There were also many filamentous bacteria and large protozoans in the activated sludge.

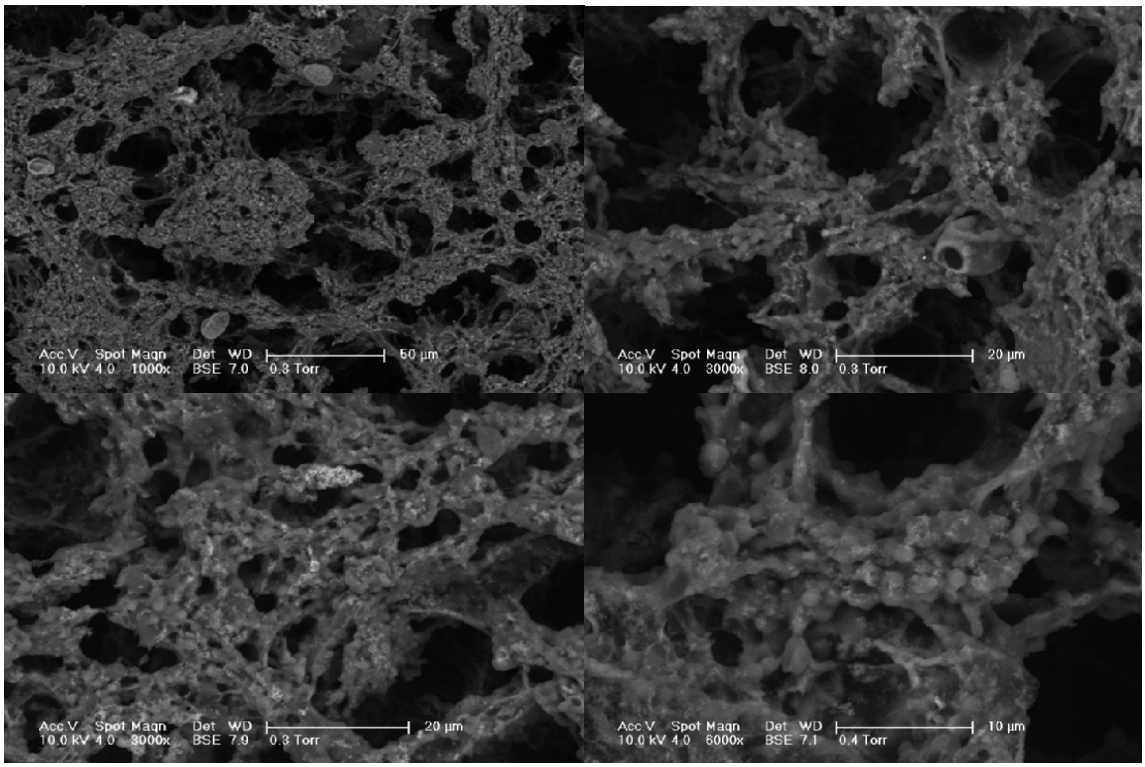


Figure 3.3. Environmental scanning electron microscopy of microorganisms and flocs in non-acclimated activated sludge taken from the mother reactor.

A mineral salts solution was prepared and added to the reactors to provide the necessary nutrients for microorganisms. The stock mineral salts solution used in biodegradation and bioregeneration experiments was composed of 5000 mg/L $(NH_4)_2SO_4$, 2000 mg/L KH_2PO_4, 2000 mg/L K_2HPO_4, 2000 mg/L $MgSO_4.7H_2O$, 450 mg/L $CaCl_2$, 200 mg/L $FeCl_3.6H_2O$, and 3000 mg/L $NaHCO_3$. This solution was suitable for a wastewater with a COD of 10000 mg/L and was diluted in biodegradation and bioregeneration reactors (shown in Figure 3.2) in proper amounts to compensate for the nutrient requirement. Throughout the biodegradation and bioregeneration experiments with phenol, it was used as the sole carbon source in reactors.

In batch bioregeneration studies, activated carbon was initially loaded with phenol by contacting activated carbon with excessive phenol (1000 mg) and agitating until equilibrium was reached. The supernatant was removed by centrifugation. Then, by measuring the amount of phenol remaining in the supernatant, the loading on the activated carbon was calculated (loading amounts can be seen in Table 3.3). Loaded activated

carbon was mixed with 1 L activated sludge taken from the mother reactor and a proper amount of mineral salts was added. Biodegradation reactors were operated in parallel to the bioregeneration reactors. The control biodegradation reactors were fed with an amount of phenol (Table 3.3) equal to the amount of phenol loading on the activated carbon in the bioregeneration reactor. Also a proper amount of mineral salts and 1 L activated sludge were added to the biodegradation reactors as in bioregeneration reactors. The bulk phenol and COD concentrations in the reactors were measured with respect to time. Oxygen uptake rate (OUR) measurements were conducted to provide useful data for the determination of bioregeneration. The operational conditions in biodegradation and bioregeneration reactors in seven runs are shown in Table 3.3. The pH values in the bioregeneration reactors ranged between 7-7.5 during the first few hours of aeration and decreased gradually to 5.5-6 at the end of 72 hours.

Table 3.3. Main characteristics of biodegradation and bioregeneration experiments with phenol as the target compound.

Run	Reactor Type	Activated Carbon	Carbon Characteristics	Carbon Dose (mg/L)	Initial phenol loading (mg /g)	Initial Bulk Phenol (mg/L)	Initial MLSS (mg/L)
1	Biodegradation	-	-	-	-	163.4	3165
	Bioregeneration	SA4	Powdered, thermally activated	1000	168.5	-	3165
2	Biodegradation	-	-	-	-	290	2325
	Bioregeneration	SA4	Powdered, thermally activated	2000	161.4	-	2325
3	Biodegradation	-	-	-	-	261	2295
	Bioregeneration	CA1	Powdered, chemically activated	2000	137.5	-	2295
4	Biodegradation	-	-	-	-	311.5	1865
	Bioregeneration	PKDA	Granular, thermally activated	2000	170	-	1865
5	Biodegradation	-	-	-	-	264.4	1400
	Bioregeneration	CAgran	Granular, chemically activated	2000	140.25	-	1400
6	Bioregeneration	SA4	Powdered, thermally activated	2000	194.25	-	1558
	Bioregeneration	CA1	Powdered, chemically activated	2000	174.25	-	1558
7	Bioregeneration	PKDA	Granular, thermally activated	2000	172.25	-	1056
	Bioregeneration	CAgran	Granular, chemically activated	2000	162.25	-	1056

At the end of the runs or during the runs, activated carbon samples (100-500 mL) were taken from the bioregeneration reactors for post-bioregeneration adsorption experiments. These carbon samples were contacted with excessive phenol and agitated

until equilibrium as in the case of initial loading before the bioregeneration experiments. The amounts of additional phenol loadings during this post-bioregeneration adsorption were considered as the bioregenerated amounts as explained in Section 2.3.6.2.

3.4. Experiments with 2-Chlorophenol

3.4.1. Adsorption Experiments with 2-Chlorophenol

Adsorption studies with 2-chlorophenol were performed in 100 mL flasks shaken at 140 RPM and 25°C as in experiments with phenol (Section 3.3.1). Activated carbon doses of 100-1600 mg/L were contacted with fixed concentrations of 2-chlorophenol (200 mg/L) and the mixture was agitated until reaching equilibrium. The equilibrium time was determined as 7 days for SA4 and PKDA, and 1 day for CA1 and CAgran.

3.4.2. Desorption Experiments with 2-Chlorophenol

Desorption studies with 2-chlorophenol were performed with 2000 mg/L of each activated carbon in 250 mL flasks shaken at 140 RPM and 25°C as in experiments with phenol (Section 3.3.2). Desorption equilibrium for 2-chlorophenol was determined as 24 hours for each activated carbon type.

3.4.3. Biodegradation and Bioregeneration Experiments with 2-Chlorophenol

The same batch reactors and the same procedure were used for the biodegradation and bioregeneration experiments with 2-chlorophenol as in experiments with phenol (Section 3.3.3). In biodegradation and bioregeneration experiments with 2-chlorophenol the stock mineral salts solution was composed of 5000 mg/L $(NH_4)_2SO_4$, 2000 mg/L KH_2PO_4, 2000 mg/L K_2HPO_4, 2000 mg/L $MgSO_4.7H_2O$, 500 mg/L $CaSO_4.2H_2O$, 200 mg/L $FeSO_4.7H_2O$, and 3000 mg/L $NaHCO_3$. Chloride containing nutrients were not used in experiments with 2-CP in order to make reliable chloride measurements for determination of biodegradation and bioregeneration. Throughout these biodegradation and bioregeneration experiments (Table 3.4), 2-chlorophenol was used as the sole carbon source in reactors.

Table 3.4. Main characteristics of biodegradation and bioregeneration experiments with 2-chlorophenol (2-CP) as the target compound.

Run	Reactor Type	Activated Carbon	Biomass	Carbon Dose (mg/L)	Initial 2-CP loading (mg /g)	Initial Bulk 2-CP (mg/L)	Initial MLSS (mg/L)
8	Biodegradation	-	Non-acclimated	-	-	384	1200
	Bioregeneration	SA4	Non-acclimated	2000	212.5	-	1200
9	Biodegradation	-	Acclimated	-	-	139.6	550
	Bioregeneration	SA4	Acclimated	2000	74.5	-	550

SA4: Powdered, thermally activated

In biodegradation-bioregeneration RUN 8 with 2-chlorophenol, non-acclimated sludge was used as in the experiments with phenol. In RUN 9 with 2-chlorophenol, activated sludge was acclimated by feeding with phenol and 2-chlorophenol. Acclimation in a batch reactor was started with 200 mg/L phenol as the only carbon source. The 2-chlorophenol concentration in mixture was gradually increased up to 140 mg/L (with 80 mg/L phenol) in a period of 40 days. Batchwise feeding was repeated every 2-3 days. At the end of every 48-72 hour aeration period, it was observed that almost all of the phenol and 2-chlorophenol were removed and the biological activity was measured by initial oxygen uptake rates as high as 20 mg/L.h.

3.5. Experiments with a Mixture of Phenol and 2-Chlorophenol

3.5.1. Adsorption Experiments with a Mixture of Phenol and 2-Chlorophenol

A procedure similar to the one used in experiments with phenol (Section 3.3.1) was used for the adsorption isotherms of a bi-solute mixture of phenol and 2-chlorophenol. Bi-solute adsorption studies were performed in 100 mL flasks shaken at 140 RPM and 25°C. Activated carbon doses of 200-4000 mg/L were contacted with fixed concentrations of phenol and 2-chlorophenol (200 mg/L of each) and the mixture was agitated until reaching equilibrium. KH_2PO_4 (1000 mg/L) and K_2HPO_4 (1000 mg/L) were dissolved in the mixture to adjust pH to 7 and the mixture was agitated until reaching equilibrium. The equilibrium time was determined to be less than 7 days in previous single-solute adsorption

studies (Section 3.3.1 and 3.4.1). Hence, agitation time was set as 7 days for each carbon type.

3.5.2. Desorption Experiments with a Mixture of Phenol and 2-Chlorophenol

A procedure similar to the one used in experiments with phenol (Section 3.3.2) was used for the desorption isotherms of a bi-solute mixture of phenol and 2-chlorophenol. Desorption studies were performed with 2000 mg/L of each activated carbon in 250 mL flasks shaken at 140 RPM and 25°C. Activated carbon (2000 mg/L) was initially contacted with an excess amount of phenol (500 mg/L) and 2-CP (250 mg/L) and was agitated for 7 days until equilibrium was reached as in adsorption batches. KH_2PO_4 (1000 mg/L) and K_2HPO_4 (1000 mg/L) were also added to mixture both at the initial loading of carbons and at each desorption step to adjust pH to 7. Desorption equilibrium time was previously determined as 24 hours both for phenol and 2-CP (Section 3.3.2 and 3.4.2). Desorption was conducted successively until the phenol and 2-CP concentrations in the supernatant were under the gas chromatographic detection limit of about 0.2 mg/L.

3.5.3. Biodegradation and Bioregeneration Experiments with a Mixture of Phenol and 2-Chlorophenol

In biodegradation and bioregeneration experiments with a mixture of phenol and 2-chlorophenol, the same stock mineral salts solution was used as in experiments with 2-CP in Section 3.4.3. Throughout these experiments, phenol and 2-CP were used as the only carbon sources in reactors and an acclimated activated sludge was used. The activated sludge described in Section 3.3.3 was acclimated to phenol and 2-CP. The acclimated sludge used in Section 3.4.3 was continued to be acclimated with about 170 mg/L phenol and 110 mg/L 2-CP fed every two days. Acclimation was continued for more than 1 year before beginning the biodegradation and bioregeneration runs with a mixture of phenol and 2-CP. Environmental scanning electron microscopic analysis of the acclimated sludge showed that bright cocci-shaped bacteria dominated (Figure 3.4). But large protozoans and particularly filamentous microorganisms seemed to decrease in number compared with the non-acclimated sludge in Figure 3.3. Other ESEM images from the activated sludge acclimated with phenol and 2-CP can be seen in Figure B.2 in Appendix B.

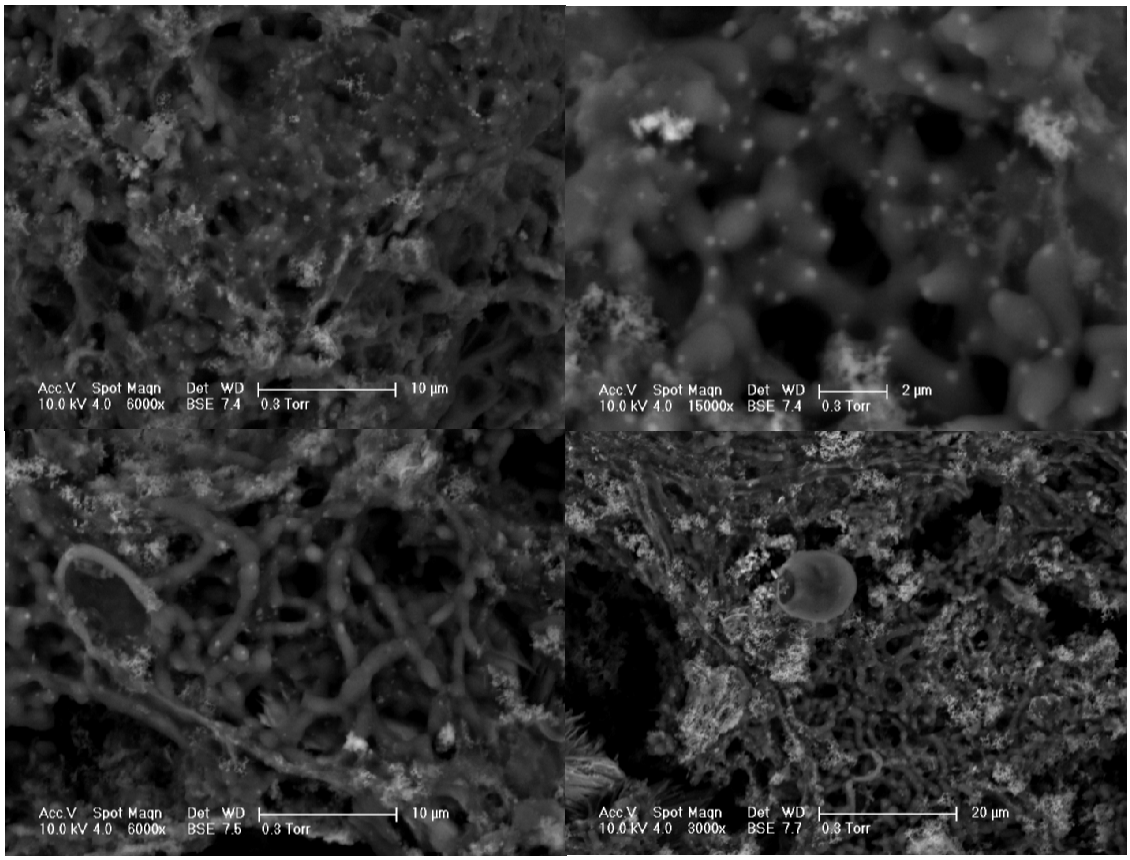


Figure 3.4. Environmental scanning electron microscopy of microorganisms in activated sludge acclimated with phenol and 2-CP.

During the initial adsorption step before bioregeneration, pH was adjusted to 7 as described in Section 3.5.1. In biodegradation and bioregeneration reactors, pH ranged between 6.94-7.77 in the bulk liquid. Acclimated sludge was added to each reactor. The initial conditions for biodegradation and bioregeneration experiments are tabulated in Table 3.5. The control biodegradation reactors were fed with an amount of phenol and 2-CP (Table 3.5) equal to the amount of phenol and 2-CP loadings on the activated carbon in the bioregeneration reactors.

At the end of the runs or during the runs, activated carbon samples (100-500 mL) were taken from the bioregeneration reactors for post-bioregeneration adsorption experiments. These carbon samples were contacted with excessive phenol and 2-CP and agitated until equilibrium as in the case of initial loading before the bioregeneration experiments. These measurements were used for quantification of bioregeneration.

In order to measure the extractable phenol and 2-CP remaining on carbon samples, solvent extraction was applied. For this purpose 250 mL to 2L mixed liquor samples (containing 500 mg to 4 g carbon sample) were taken from the bioregeneration reactors at the end of the runs. Carbon samples were separated from the supernatant through centrifugation at 4000 RPM. These carbon samples were extracted with 100 mL methylene chloride, which was also used as a solvent in gas chromatographic analysis. Extraction was performed by Velp Scientifica SER 148 solvent extractor. The solvent was placed in a bottle of the closed extraction system, which is heated by a plate under the system. The temperature of the heating plate was adjusted to 140°C, which is about 100°C above the boiling point of methylene chloride (39.4°C). Carbon samples were placed in a cylindrical thimble, and the thimble was immersed in a heated solvent for 1 hour. After immersion, the thimble containing the sample was taken out of the solvent bottle. Then the sample was washed for 1 hour with the boiling solvent. This extraction method decreased the time required for extraction by 4 –6 times. At the end of extraction, 1 mL of the extraction solvent methylene chloride was injected to the gas chromatograph for analysis.

Table 3.5. Main characteristics of biodegradation and bioregeneration experiments with a mixture of phenol and 2-chlorophenol.

Run	Reactor Type	Activated Carbon	Carbon Dose (mg/L)	Initial phenol loading (mg /g)	Initial Bulk Phenol (mg/L)	Initial 2-CP loading (mg /g)	Initial Bulk 2-CP (mg/L)	Initial MLSS (mg/L)
10	Biodegradation	-	-	-	249.7		170.4	1900
	Bioregeneration	SA4	2000	133.2	-	97.1	-	1900
11	Biodegradation	-	-	-	154.1		151	1360
	Bioregeneration	CA1	2000	102.4	-	95.6	-	1360
12	Biodegradation	-	-	-	185.3	-	152.6	1330
	Bioregeneration	PKDA	2000	82.4	-	92.2	-	1330
13	Biodegradation	-	-	-	133.8	-	145.8	1635
	Bioregeneration	CAgran	2000	79.8		81.8	-	1635
14	Bioregeneration	SA4	2000	133.2	-	97.1	-	925
	Bioregeneration	CA1	2000	102.4	-	95.6	-	925
15	Bioregeneration	PKDA	2000	99.9	-	94.5	-	865
	Bioregeneration	CAgran	2000	96.1	-	88.0	-	865

3.6. Experiments with 2-Nitrophenol

3.6.1. Adsorption Experiments with 2-Nitrophenol

Adsorption studies with 2-nitrophenol (2-NP) were performed in 100 mL flasks shaken at 140 RPM and 25°C as in experiments with phenol (Section 3.3.1). Activated carbon doses of 100-1400 mg/L were contacted with fixed concentrations of 2-nitrophenol (200 mg/L). KH_2PO_4 (1000 mg/L) and K_2HPO_4 (1000 mg/L) were dissolved in the mixture to adjust pH to 7 and the mixture was agitated until reaching equilibrium. Only powdered carbons SA4 and CA1 were used in studies with 2-NP. Equilibrium time for adsorption studies was determined as 4 days for SA4, and 7 days for CA1.

3.6.2. Desorption Experiments with 2-Nitrophenol

Desorption studies were performed with 2000 mg/L of each activated carbon in 250 mL flasks shaken at 140 RPM and 25°C. Activated carbon (2000 mg/L) was initially contacted with an excess amount of phenol (500 mg/L) and 2-NP (250 mg/L) and was agitated until equilibrium was reached as in adsorption studies. KH_2PO_4 (1000 mg/L) and K_2HPO_4 (1000 mg/L) were also added to mixture both at initial loading of carbons and at each desorption step to adjust pH to 7. Desorption equilibrium occurred in 24 hours. Desorption was conducted successively until the 2-NP concentrations in the supernatant were under the gas chromatographic detection limit of about 0.2 mg/L.

3.6.3. Biodegradation and Bioregeneration Experiments with a Mixture of Phenol and 2-Nitrophenol

In biodegradation and bioregeneration experiments with a mixture of phenol and 2-nitrophenol, the same stock mineral salts solution was used as in experiments with 2-CP in Section 3.4.3. Throughout these biodegradation and bioregeneration experiments, phenol and 2-NP were used as the only carbon sources in reactors except in the biodegradation reactor of RUN 18 (Table 3.6). During the initial adsorption step before bioregeneration, pH was adjusted to 7 as described in Section 3.6.1. In biodegradation and bioregeneration reactors, pH ranged between 7.2-7.7 in the bulk liquid. Acclimated sludge was added to

each reactor except one biodegradation reactor in RUN 16 (Table 3.6). The initial conditions for biodegradation and bioregeneration experiments are tabulated in Table 3.6. Activated carbon samples were taken at the end of bioregeneration runs for post-bioregeneration adsorption experiments and solvent extractions as in Section 3.5.3.

Table 3.6. Main characteristics of biodegradation and bioregeneration experiments with a mixture of phenol and 2-nitrophenol.

Run	Reactor Type	Activated Carbon	Carbon Dose (mg/L)	Initial phenol loading (mg /g)	Initial Bulk Phenol (mg/L)	Initial 2-NP loading (mg /g)	Initial Bulk 2-NP (mg/L)	Initial MLSS (mg/L)
16	Biodegrad. (Acc.AS)*	-	-	-	200	-	60	970
	Biodegrad. (Non.Acc.AS)*		-	-	200	-	60	2740
17	Biodegradation	-	-	-	200	-	100	962
	Bioregeneration	SA4	2000	48.2	-	160.5	-	962
18	Biodegradation	-	-	-	-	-	100	614
	Bioregeneration	CA1	2000	95.7	-	224.1	-	614
19	Bioregeneration	SA4	2000	82.8	-	51.6	-	450
	Bioregeneration	CA1	2000	56.4	-	48.6	-	450

*Acc. AS: Acclimated activated sludge
Non-Acc.AS: Non-acclimated activated sludge

In these experiments, an acclimated activated sludge was used. The activated sludge described in Section 3.3.3 was acclimated to phenol and 2-NP. Acclimation in a batch reactor was started with 200 mg/L phenol as the only carbon source. 2-Nitrophenol in mixture was increased gradually up to 60 mg/L (with 200 mg/L phenol) in a period of 4 months. Batchwise feeding was repeated every 2-3 days. At the end of every 48-72 hour aeration period, almost all of the phenol and 2-NP were removed and the biological activity measured by initial oxygen uptake rates was as high as 45 mg/L.h. Acclimation was continued for more than 4 months before beginning the biodegradation and bioregeneration runs with a mixture of phenol and 2-NP. RUN 16 (Table 3.6) was performed to investigate the effect of acclimation on bi-solute biodegradation of phenol and 2-NP. ESEM analysis of the acclimated sludge showed that cocci-shaped bacteria and large protozoans dominated (Figure 3.5). But, filamentous microorganisms seemed to decrease in number compared with the non-acclimated sludge in Figure 3.3. Other ESEM images from the activated sludge acclimated with phenol and 2-NP can be seen in Figure B.3 in Appendix B.

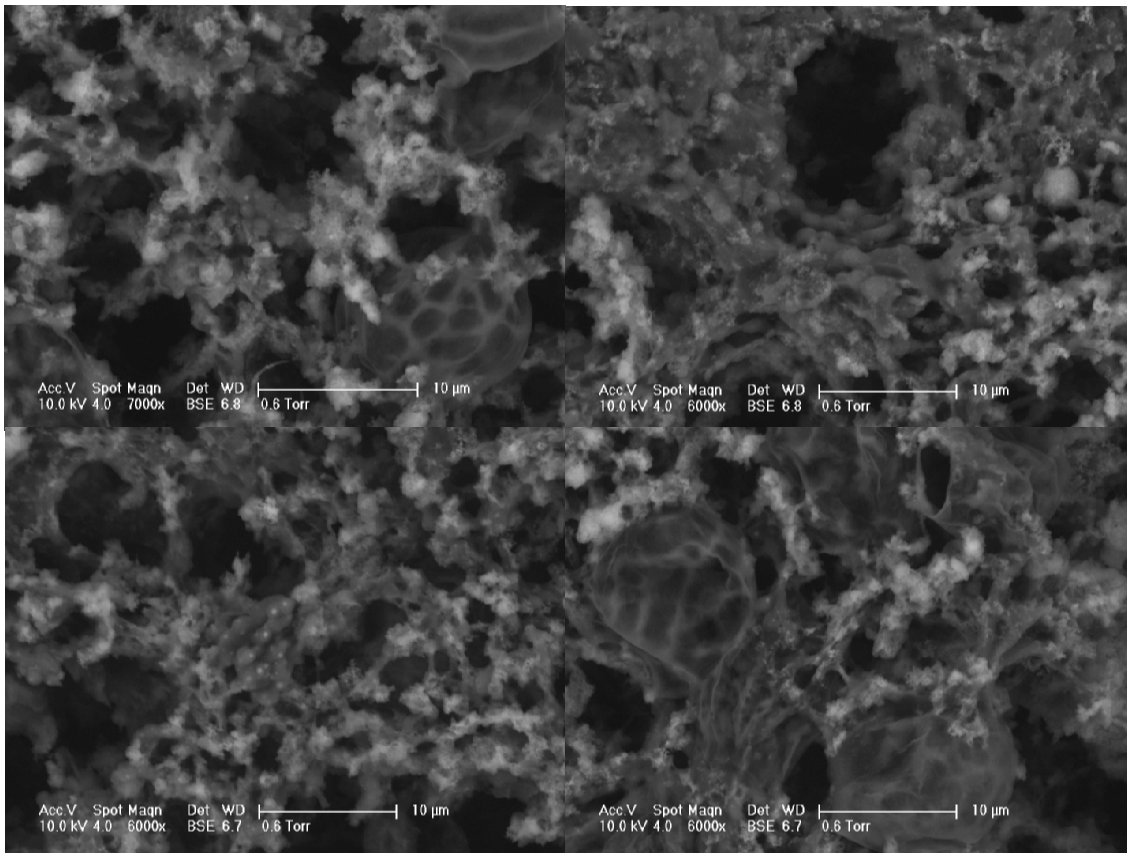


Figure 3.5. Environmental scanning electron microscopy of microorganisms in activated sludge acclimated with phenol and 2-NP.

3.7. Analytical Methods

The analytical methods used in the experiments followed the *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WPCF, 1998) except gas chromatographic measurements. Analytical methods are explained below:

a) COD Analysis: For chemical oxygen demand (COD) analysis the dichromate closed reflux method (APHA-AWWA-WPCF, 1998) was employed. The soluble COD (SCOD) of the final liquids were determined after filtering through 0.45 µm Millipore filters to remove microorganisms, particulate microbial products and activated carbon particles. Samples were refluxed with potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4) for two hours at 150°C in the Hach COD digester, in the presence of Ag_2SO_4 as catalyst and $HgSO_4$ to prevent chloride interference. High range COD measurements

were done colorimetrically by measuring the absorbance of samples at 600 nm with a Hach DR/2010 Spectrophotometer. COD values below 80 mg/L were measured by the low range method. Low range COD measurements were also done colorimetrically, but by measuring the absorbance of samples at 420 nm. In low range COD method, diluted potassium dichromate solution was used and distilled water was used as blank without addition of dichromate solution and sulfuric acid solution. On the other hand, distilled water treated with the dichromate solution and sulfuric acid was used as reference and the difference between the absorbance of reference and sample was proportional to the COD of sample. Calibration curves for high range and low range methods (Figures A.1 and A.2 in Appendix A) were prepared by using standard KHP (Potassium Hydrogen Phthalate) solutions with correctly estimated COD values.

b) Measurement of phenol and 2-chlorophenol by the 4-Aminoantipyrine method: Phenol and 2-chlorophenol concentrations were measured colorimetrically according to the 4-aminoantipyrine method (APHA-AWWA-WPCF, 1998) in Sections 3.3 and 3.4, when they were the only phenolic compounds in the solution. This method is applicable with high accuracy and precision when there is a unique phenolic species (Neufeld and Paladino, 1985). Samples were filtered through 0.45 μm Millipore filters before every measurement. Samples were diluted to 100 mL to result in phenol concentrations less than 5 mg/L. Two calibration curves (Figures A.3 and A.4 in Appendix A) were prepared with phenol standards. These curves included the concentrations between 0.1-1 mg/L and between 1-5 mg/L. Each measurement required the use of a distilled water blank. Standards, blank and each sample were treated by adding 0.5N NH_4OH solution to increase pH above 10, and pH was adjusted immediately to 7.9±0.1 with phosphate buffer. After addition of 4-aminoantipyrine and $K_3Fe(CN)_6$ solutions a colored complex formed. The absorbance of sample and standards were read against the blank at 500 nm with a Hach DR/2010 spectrophotometer.

c) Gas Chromatographic (GC) analysis: Phenol and 2-CP concentrations in bi-solute experiments (Section 3.5) and all 2-nitrophenol concentrations (Section 3.6) were determined with an Agilent 6890N gas chromatograph equipped with an FID (Flame Ionization detector) and HP-5 column (length 30m, ID 0.32 mm, film thickness 0.25μm). Samples were filtered through 0.45 μm Millipore filters before every measurement.

Helium was used as the carrier gas at the splittless mode with a flow rate of 25 cm/s. The inlet temperature was 240°C and detector temperature was 300°C. The oven temperature was held at 40°C for 1 min, increased to 140°C at 10°C/min and then increased to 260°C at 20°C/min. 1 mL sample was extracted with 0.5 mL methylene chloride in 2 mL closed vials for 3 minutes and 2 μL of the methylene chloride phase was injected with an Agilent 7683 auto-injector. Standard solutions containing 5-200 mg/L of phenol, 2-chlorophenol and 2-nitrophenol were used to prepare calibration curves (Figure A.5 in Appendix A). Concentrations were measured using the total area under each peak together with the calibration curves. Figure A.6 in Appendix A shows samples of peaks. Also, a mixed standard (Ultra Scientific PHM-604 phenols mixture) of phenolic compounds was used to check the accuracy of the calibration curves. The chromatogram obtained for the mixed standard is shown in Figure A.7 in Appendix A. The GC method separated individual phenolics and thus could provide greater accuracy than the 4-aminoantipyrine method (Neufeld and Paladino, 1985).

d) Chloride Analysis: Chloride concentrations were determined according to the argentometric method (APHA-AWWA-WPCF, 1998). Samples were initially filtered through 0.45 μm Millipore filters and potassium dichromate ($K_2Cr_2O_7$) was added to each sample. Mixture was titrated with a 0.0132 N $AgNO_3$ solution. Chloride concentrations were measured considering use of one mole of titrant equals one mole of chloride in solution

e) Oxygen Uptake Rates (OUR) determination: OURs in the activated sludge reactors were measured automatically by WTW Microprocessor Oximeter Oxi 3000. OUR (mgO_2/L/h) measurement is based on the dissolved oxygen consumption in the 300 mL BOD bottle. Sample taken from the activated sludge reactor is placed in a BOD bottle and stirred with a magnetic stirrer. Dissolved oxygen concentration in the BOD bottle declines with respect to time. The slope of the dissolved oxygen decline curve gives OUR. Oximeter Oxi 3000 calculates the slope automatically and gives the OUR value. Oximeter Oxi 3000 is very sensitive to low dissolved oxygen concentrations. It does not calculate OUR values if the dissolved oxygen concentration drops to below 1 mg/L, because OUR measurements are not reliable below 1 mg/l. Below 1 mg/l, the dissolved oxygen concentration becomes the rate limiting substrate and the activity of microorganisms decreases.

f) Environmental Scanning Electron Microscopy (ESEM) analyses: ESEM images were obtained by a Philips XL30-FEG Environmental SEM operating at the wet mode. Environmental SEM analyses eliminated the requirement of sample preparation, which is necessary in conventional SEM. In ESEM analysis in Section 4.1, samples were placed in the microscope in the form of a concentrated solution and the temperature was applied as 1°C to prevent the microorganisms from high temperatures caused by the electron beams in the micro-climatic vicinity. However, this method resulted in death of most of the microorganisms. In ESEM analysis in Section 3 and 4.3, samples were placed in the microscope after filtering through 0.45 μm Millipore filters and the temperature was applied as 25°C and pressure was 0.3-0.6 Torr. This method gave much better views of microorganisms. Magnification was up to 15000 times.

g) Mixed Liquor Suspended Solid (MLSS) analysis was carried out by drying the residue on 0.45 μm Millipore filters for one hour at 103 ^{o}C.

h) pH Analysis: Hanna 211 microprocessor pH meter was used.

4. RESULTS AND DISCUSSION

4.1. Experiments with Phenol as a Model Compound

In the first part of the experimental work, phenol was used as the model compound because it is known to be both biodegradable and adsorbable on activated carbon.

4.1.1. Adsorption Studies with Phenol

The adsorption isotherm data are plotted in Figure 4.1**.** The adsorption characteristics of activated carbons were investigated using the Freundlich model in adsorption isotherms. The Freundlich equation given below is very widely used for activated carbon applications.

$$q = K\ S_e^{1/n} \tag{4.1}$$

q = adsorption capacity of the activated carbon (mg phenol adsorbed per g activated carbon)

S_e = the equilibrium phenol concentration (mg/L).

K and 1/n are the Freundlich constants (Freundlich exponent and slope).

Table 4.1. Freundlich isotherm constants for phenol adsorption.

Carbon type	**Physical form**	**Activation method**	**K [(mg/g)(L/mg)$^{1/n}$]**	**1/n**	**R^2**
Norit SA4	Powdered	Thermal	67.6	0.1830	0.90
Norit CA1	Powdered	Chemical	6.3	0.4814	0.93
Norit PKDA	Granular	Thermal	60.7	0.1866	0.85
Norit CAgran	Granular	Chemical	18.4	0.2294	0.86

Freundlich constants obtained by regression analysis are shown in Table 4.1. The K value, which is an indicator of adsorption capacity, was found to be higher for the thermally activated carbons compared with the chemically activated carbons (Table 4.1). The thermally activated carbons were obviously better adsorbers for phenol although the

total surface areas of chemically activated carbons were much higher than thermally activated carbons (Table 3.1). The adsorption performance is dependent on the condition of internal surface accessibility (Martin et al., 2002) and chemical surface characteristics of the adsorbent (Jonge et al., 1996b), and not on total surface area. The adsorption intensity, 1/n, was shown by the slope of the adsorption isotherm curves (Figure 4.1). Lower 1/n values for the thermally activated carbons indicate that an increase in the activated carbon dose was more effective for thermally activated carbons rather than in the case of chemically activated carbons with steeper isotherm curves. This effect can be seen more clearly in Figure 4.2, which illustrates the effect of carbon dose on bulk equilibrium phenol concentrations. Also in other studies conducted with trichloroethylene and trichlorobenzene (Karanfil and Kilduff, 1999), and o-cresol and 3-chlorobenzoic acid (Jonge et al., 1996b), adsorption capacities of thermally activated carbons were higher than chemically activated ones.

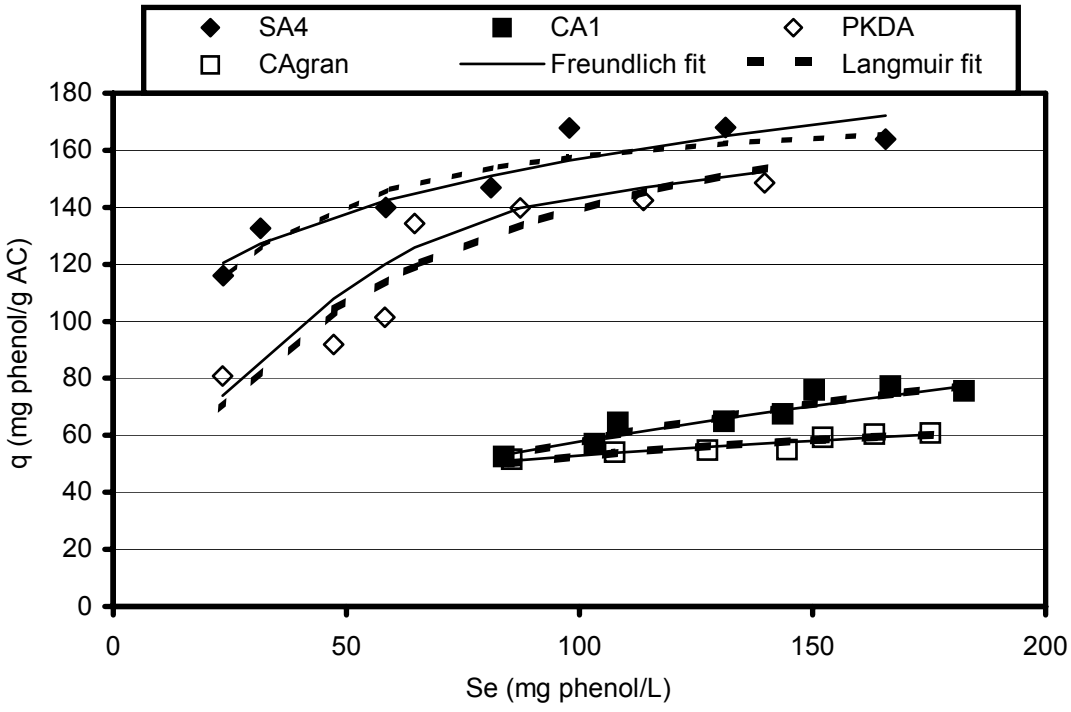


Figure 4.1. Phenol adsorption isotherms for the powdered carbons SA4 (thermally activated) and CA1 (chemically activated) and granular carbons PKDA (thermally activated) and CAgran (chemically activated) with their Freundlich and Langmuir fits.

Figure 4.2 shows that the powdered and granular countertypes (SA4-PKDA and CA1-CAgran) do not significantly differ in terms of phenol adsorption, but there are important statistical differences ($p<0.01$ at a confidence level of 95% with paired t-test) between the thermally (SA4 and PKDA) and chemically activated carbons (CA1 and CAgran). Although the granular activated carbons have higher total pore volumes (Table 3.1) than their powdered countertypes, the adsorption characteristics were similar for both powdered-granular countertypes. However, the difference in pore volume between the powdered and granular countertypes is largely due to the higher macropore volume of granular carbons. This shows that phenol adsorption on the macropores was much less significant compared with meso- and micropores. It should be added that macropores do not adsorb small molecules like phenol by volume but by surface. Considering that most of the total surface area is found in the micropores and contribution of macropores to total surface area is very low, this is considered to be an expected result. Also, higher sorption energy in micropores makes them more available adsorption sites for relatively small molecular weight organics (Karanfil and Dastgheib, 2004).

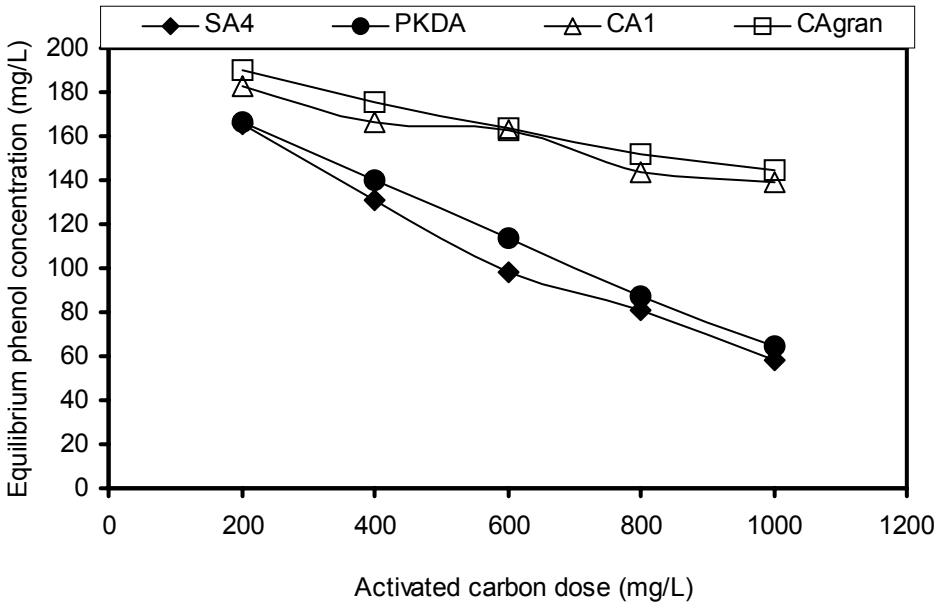


Figure 4.2. Effect of activated carbon dose on equilibrium phenol concentrations with an initial phenol concentration of 200 mg/L.

The isotherm curves tended to reach saturation as the equilibrium concentrations increased (Figure 4.1). The theoretical Langmuir equation was also tested for the adsorption isotherms. Langmuir expression is defined as in Equation 4.2:

$$q = \frac{Q°bC}{(1 + bC)} \tag{4.2}$$

in which C is the measured concentration in solution at equilibrium, Q° is the number of moles of solute adsorbed per unit weight of adsorbent in forming a complete monolayer on the surface, q is the number of moles of solute adsorbed per unit weight at concentration C, and b is a constant related to the energy of adsorption. The linear form of the Langmuir equation is given in Equation 4.3. From the slope and intercept b and Q° were calculated (Table 4.2).

$$\frac{1}{q} = \frac{1}{Q°} + \left(\frac{1}{bQ°}\right)\left(\frac{1}{C}\right) \tag{4.3}$$

Table 4.2. Langmuir isotherm constants for phenol adsorption.

Carbon type	**Physical form**	**Activation method**	**Q° (mg/g)**	**b (L/mg)**	**R^2**	**R_L**
Norit SA4	Powdered	Thermal	178.6	0.0763	0.99	0.268
Norit CA1	Powdered	Chemical	129.9	0.0081	0.90	0.826
Norit PKDA	Granular	Thermal	204.1	0.0219	0.96	0.528
Norit CAgran	Granular	Chemical	74.1	0.0248	0.98	0.731

Relatively high R^2 values were also obtained for the Langmuir equation indicating an acceptable correlation between the adsorption isotherm data and the Langmuir equation (Table 4.2 and Figure 4.1). Higher Q° values obtained for the two thermally activated carbons SA4 and PKDA indicated higher adsorption capacities for these carbons compared with chemically activated carbons as also evidenced from the Freundlich equation. The essential characteristics of the Langmuir equation can be expressed (Al-Degs et al., 2000) in terms of a dimensionless separation factor R_L which is defined by equation below:

$$R_L = 1/(1 + Q° \times b \times C_o) \tag{4.4}$$

where C_o is the initial phenol concentration (g/L).

The value of R_L indicates the shape of the isotherm to be either unfavorable ($R_L>1$), linear ($R_L=1$), favorable ($0<R_L<1$) or irreversible ($R_L=0$). The R_L values for each activated carbon (Table 4.2) were found to be between 0 and 1 indicating a favorable adsorption of phenol on each carbon type. The adsorption of phenol on two thermally activated carbons was found to be more favorable and closer to the irreversible adsorption edge of $R_L=0$ as evidenced from relatively lower R_L values (Al-Degs et al., 2000). This finding is in agreement with the results of subsequent desorption studies. Hence, by using the dimensionless separation factor R_L from the Langmuir equation, adsorption isotherms can be successfully used to have an idea on the reversibility of adsorption and to make comparisons.

Although the total surface area and total pore volumes (also both micro- and mesopore volumes) of chemically activated carbons CA1 and CAgran are higher than thermally activated carbons SA4 and PKDA (Table 3.1), adsorption capacities of thermally activated carbons were relatively higher. This was certainly caused by their activation method, which eventually results in different surface characteristics. In literature, it is generally believed that the heterogeneity of the activated carbon surface significantly contributes to its adsorption capacity (Ahnert et al., 2003). Heterogeneity of the carbon surface is particularly due to surface oxygen groups which, although present in relatively small amounts, affect surface properties such as surface acidity, polarity or hydrophobicity, and surface charge. The presence of heterogeneous oxygen groups on the carbon surface is known to reduce the adsorption capacity due to water adsorption on these groups via hydrogen bonding (Ahnert et al., 2003). It has been stated that increasing the number of oxygen-containing surface functional groups increases the polarity of carbon surfaces. Therefore, their selectivity for water increases and thus adsorbed water clusters may block carbon pores (Karanfil and Kilduff, 1999). Chemical treatment of activated carbon increases the quantity of acidic surface functional groups, whereas thermal treatment results in a decrease in the number of acidic surface functional groups (Julien et al., 1998).

In this present study, equilibrium pH values obtained in adsorption isotherm studies pointed out that the surface characteristics of the two thermally activated carbons SA4 (pH between 7.2-8.7 depending on the carbon dose) and PKDA (pH between 7.5-8.8) were basic, and surface characteristics of the two chemically activated (with phosphoric acid)

carbons CA1 (pH between 3.2-5.0) and CAgran (pH between 5.2-5.9) were acidic. It has to be noted that the differences in pH values are due to acidic or basic surface functional groups that could be freed by simple contact with distilled water rather than the fixed surface functional groups. An inverse relationship has previously been reported (Karanfil and Kilduff, 1999; Ahnert et al., 2003) between adsorption capacity and surface acidity, or equivalently polarity since polarity generally correlates with the number of acidic groups. Since water adsorption is expected to occur on polar adsorption sites (Julien et al., 1998), more water adsorption is expected to occur on chemically activated carbons with acidic surface properties rather than thermally activated carbons. Chemically activated carbons provide a pore surface which is less hydrophobic and more negatively charged. This results eventually in lower adsorption capacities for chemically activated carbons CA1 and CAgran in this present study.

4.1.2. Desorption Studies with Phenol

Desorption (Figures 4.3 and 4.4) data for each carbon type were fitted into the Freundlich type isotherm equation with satisfactory correlations. Desorption Freundlich isotherm constants are shown in Table 4.3. High K values for thermally activated carbons SA4 and PKDA showed that phenol loading was still high after desorption, therefore it can be deduced that the desorbability from thermally activated carbons is lower. Desorption capacities are higher for chemically activated carbons CA1 and CAgran. Also lower 1/n values in the case of thermally activated carbons indicate that desorption is more difficult from these carbons.

The isotherm results (Table 4.3) and the adsorption-desorption efficiencies shown in Table 4.4 reveal that adsorption is highly irreversible for thermally activated carbons, and highly reversible for chemically activated carbons. Reversibility of adsorption can be expressed (Ha and Vinitnantharat, 2000) as a degree of hysteresis (w) by using Equation 4.5.

$$w(\%) = \left(\frac{1/n_{ads}}{1/n_{des}} - 1 \right) x100 \qquad (4.5)$$

where $1/n_{des}$ and $1/n_{ads}$ are the desorption and adsorption intensities obtained from Freundlich isotherms, respectively. The 1/n value in desorption isotherms being smaller than the 1/n value in adsorption isotherms implies that desorption is slower (Ha and Vinitnantharat, 2000) or more difficult than adsorption. Hence, it can be stated that the degree of hysteresis is an indicator for adsorption reversibility, which bases on the difference between the adsorption and desorption intensities. Apparently, higher degrees of hysteresis (higher irreversibility) were calculated for thermally activated carbons compared with chemically activated ones (Table 4.4), and this agrees well with the findings of the isotherms and the calculated desorption efficiencies.

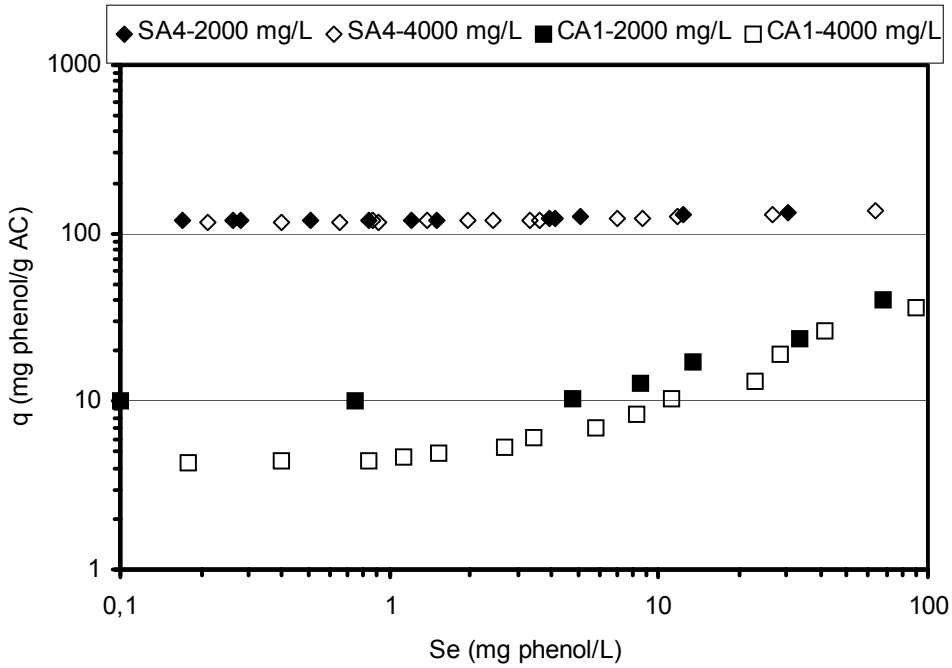


Figure 4.3. Phenol desorption isotherms for the powdered carbons SA4 (thermally activated) and CA1 (chemically activated) at doses of 2000 mg/L and 4000 mg/L .

High irreversibility of adsorption implies that the dominant adsorption mechanism is the high-energy bonding to specific functional groups on the activated carbon surface. Direct reaction of sorbate molecules with the active sites on the carbon surface results in chemisorption which is the most logical explanation for irreversible adsorption (Yonge et al., 1985). Hence, it can be stated that chemisorption is the dominant adsorption mechanism for thermally activated carbons, whereas physisorption is dominant in chemically activated carbons. The presence of hysteresis becomes also obvious when adsorption and desorption isotherm curves are considered. The differences between the

adsorption (Figure 4.1) and desorption isotherms (Figures 4.3 and 4.4) are due to irreversible adsorption as stated in the literature (Yonge et al., 1985). The findings of our study show that phenol adsorption is not always reversible, contrary to the findings of earlier studies (Schultz and Keinath, 1984), and there may be a significant hysteresis, particularly for thermally activated carbons.

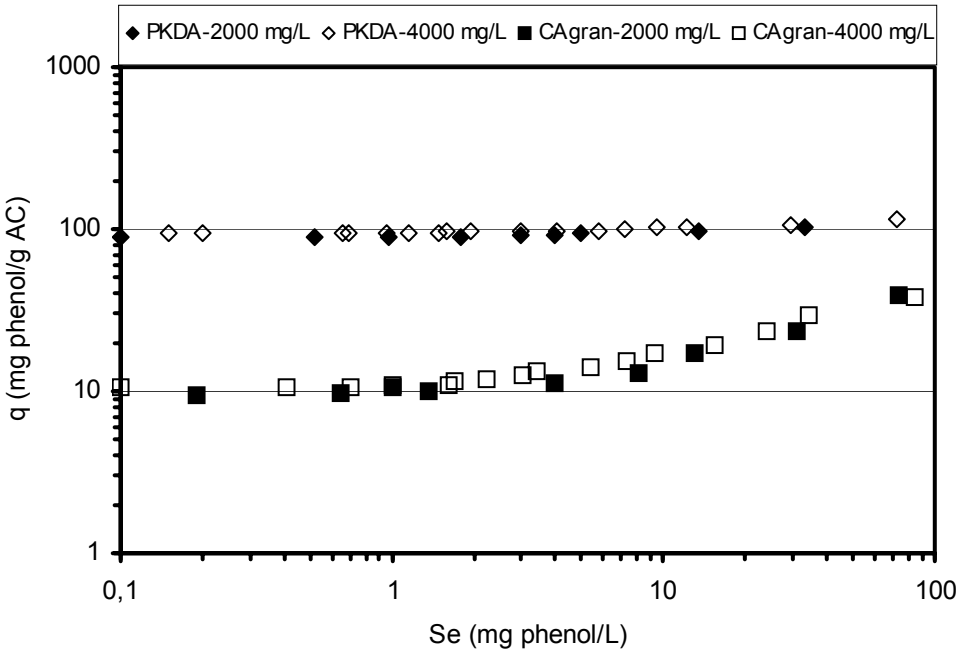


Figure 4.4. Phenol desorption isotherms for the granular carbons PKDA (thermally activated) and CAgran (chemically activated) at doses of 2000 mg/L and 4000 mg/L.

Table 4.3. Conditions of preliminary adsorption preceding desorption experiments and Freundlich desorption isotherm constants for phenol.

Carbon type	Carbon Conc. (mg/L)	Initial phenol (mg/L)	Equilib. phenol (mg/L)	Loading (mg/g)	K [(mg/g)(L/mg)$^{1/n}$]	1/n	R^2
SA4	2000	473.3	174.6	149.35	120.5	0.0242	0.85
SA4	4000	956.3	352.9	150.85	117.0	0.0280	0.91
CA1	2000	473.3	324.1	74.60	8.3	0.3070	0.81
CA1	4000	956.3	565.4	97.72	4.2	0.4509	0.92
PKDA	2000	473.3	232.6	120.35	89.3	0.0358	0.89
PKDA	4000	956.3	426.1	132.55	93.5	0.0413	0.94
CAgran	2000	473.3	321.7	75.80	9.1	0.2158	0.87
CAgran	4000	956.3	546.2	102.52	9.4	0.3094	0.95

Table 4.4. Adsorption and desorption efficiencies of phenol for each activated carbon type.

Carbon type	Carbon Conc. (mg/L)	Adsorption Efficiency (%)	Desorption Efficiency (%)	Irreversible adsorption (%)	Degree of hysteresis w (%)
Norit SA4	2000	63.1	20.3	79.7	656±268
Norit SA4	4000	63.1	22.2	77.8	554±180
Norit CA1	2000	31.5	86.6	13.4	57±80
Norit CA1	4000	40.9	95.6	4.4	7±27
Norit PKDA	2000	50.9	25.8	74.2	421±241
Norit PKDA	4000	55.4	28.7	71.3	352±160
Norit CAgran	2000	32	87.5	12.5	6±47
Norit CAgran	4000	42.9	89.8	10.2	-26±19

Degrees of hysteresis, or in other words nonsingularity, were found to be higher for powdered activated carbons SA4 and CA1 than their respective granular countertypes, PKDA and CAgran (Table 4.4). This showed that desorption is more difficult from PAC compared with its countertype GAC. The reason is that the ratio of macropores is higher within the GAC particles compared with their powdered countertypes (Table 3.1). GAC is milled away for production of PAC and some of the macropores are lost during this process. Since desorption is expected to be easier from macropores compared with meso- and micropores with greater adsorption energies (Karanfil and Dastgheib, 2004), desorption from the GAC particles was easier. But this effect should be limited since adsorption on macropores was very low because macropores adsorb phenol by surface area and contribution of macropores to the total surface area is limited. Therefore, it was likely that some phenol molecules were located in the macropores without actually being adsorbed resulting in such an ease of desorption. Moreover, degree of hysteresis was found to be negative at the dosage of 4000 mg/L CAgran indicating that desorption might be easier than adsorption in that specific case (Table 4.4). Also statistical errors obtained for w (%) values pointed out that degree of hysteresis may be negative for both chemically activated carbons (CA1 and CAgran) at each carbon dose (Table 4.4).

However, the desorption equilibrium curves (Figure 4.5) showed that during the first 5 hours of desorption, more phenol was desorbed from PACs compared with their GAC countertypes. Hence, desorption was faster from PACs, probably due to higher intraparticle diffusivity through PAC compared with GAC with a higher diameter. The differences

between PAC and GAC can certainly be attributed to the effect of particle size. Since GAC particles are much larger than PAC particles, diffusive transport into GAC is slower (Speitel and Digiano, 1987). Considering that in both thermally and chemically activated carbons the PAC-GAC countertypes resulted in comparable desorption at equilibrium, it can be said that diffusivity controls only the desorption rate, but not the total desorbability.

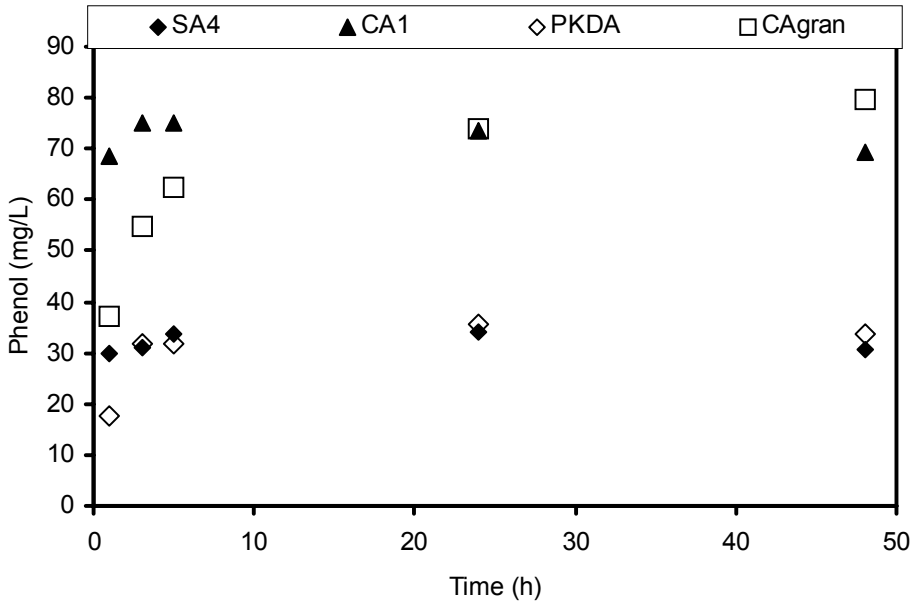


Figure 4.5. Desorption of phenol from the four loaded activated carbons with respect to time.

Figure 4.6 shows the cumulative desorbed phenol at the end of each equilibrium desorption step in desorption isotherm studies and reflects that comparable desorption is achieved from PAC-GAC countertypes. Particularly, chemically activated carbons CA1 and CAgran at doses of 2000 mg/L showed statistically similar cumulative desorption ($p>0.05$ at 95% confidence level). However, slightly higher desorption (Figure 4.6) was obtained for the GACs, particularly for PKDA, compared with their powdered countertypes due to their more macroporous structure (Table 3.1). The difference in cumulative desorption between the thermally activated carbons SA4 and PKDA was statistically significant ($p<0.01$ at 95% confidence level). Since phenol was mainly adsorbed on micro- and mesopores, the difference in macropore volume of PAC and GAC countertypes resulted in only a small difference in desorption.

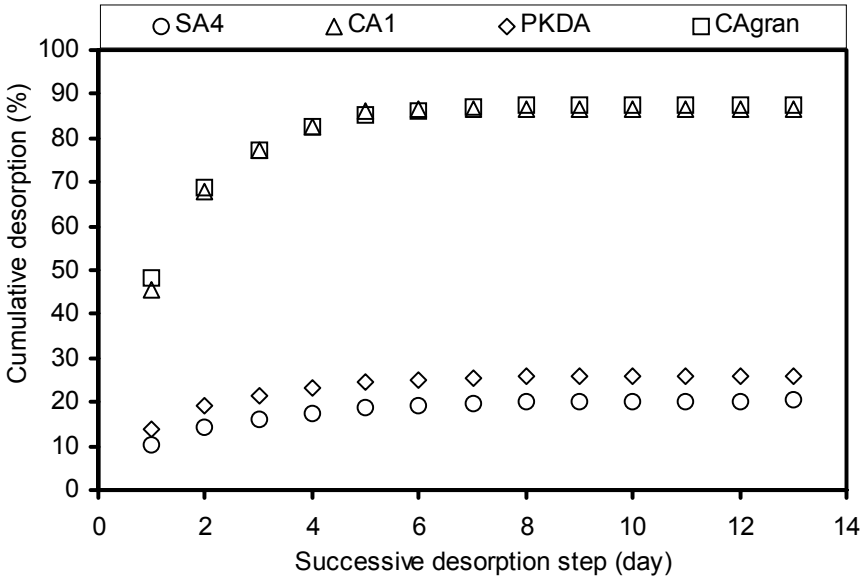


Figure 4.6. Cumulative desorption of phenol from loaded activated carbons at the end of each successive desorption step.

The results showed that higher activated carbon dosages of 4000 mg/L resulted in lower degree of hysteresis (irreversibility) compared with a lower dose of 2000 mg/L (Table 4.4). For the carbons used in this study, reversibility of adsorption increased with the carbon dose. This is contrary to an earlier study with another GAC in which reversibility of adsorption decreased with increasing carbon dose (Ha and Vinitnantharat, 2000). In that study, a fixed concentration of adsorbate was used and the resulting bulk concentrations were smaller at increased carbon doses. However, in the present study, in the preliminary adsorption stage of phenol, the initial phenol concentration at the carbon dosage of 4000 mg/L was two times higher than at the dose of 2000 mg/L. Thus, the initial phenol/carbon ratio was kept constant (236.65 mg phenol per g carbon at carbon dose of 2000 mg/L and 239.08 mg phenol per g carbon at carbon dose of 4000 mg/L). Therefore, at the activated carbon dose of 4000 mg/L, the equilibrium bulk phenol concentrations were always higher (Table 4.3). This resulted in higher phenol loading in terms of mg adsorbed phenol/g carbon (Table 4.3). When desorption took place, the amount of phenol exceeding that adsorbed at the dose of 2000 mg/L was desorbed from the carbon in a much easier way. Thus, reversibility seemed to increase with carbon dose due to the preliminary adsorption conditions of our study. This finding was obviously due to the significant change in the overall concentration range over which the hysteresis effect was quantified.

In literature it is stated that the sorption energy onto micropores is greater than onto meso- or macropores (Karanfil and Dastgheib, 2004). In the present study, this excess phenol at the carbon dose of 4000 mg/L should be certainly adsorbed with a smaller energy onto meso- or macropores. This finding of our study combined with the results in the literature (Ha and Vinitnantharat, 2000), which are not contradictory to our results, showed that in the reversibility of adsorption, the adsorption energies onto different pores plays an important role rather than the carbon dose.

The basic findings obtained in Sections 4.1.1 and 4.1.2 have been published as a paper (Aktaş and Çeçen, 2006a).

4.1.3. Biodegradation and Bioregeneration Studies with Phenol

4.1.3.1. Phenol and COD Removals. Biodegradation studies showed that phenol was completely removed or converted into nonbiodegradable or slowly biodegradable organics within 48-72 hours of aeration (Figures 4.7a-4.11a). Although phenol was completely removed after aeration, there was still soluble COD in the bulk liquid (30-50 mg/L), which may stem from side-products of phenol removal or soluble microbial products (SMP). The residual COD in RUN 1 (66 mg/L) was higher than the residual COD in RUNS 2-5 (30-50 mg/L) although initial COD was much lower in RUN 1 (Figures 4.7a-4.11a). Higher initial MLSS and lower initial phenol in RUN 1 (Table 3.3) should have resulted in higher residual COD mainly due to formation of higher SMP (soluble microbial products). The S_o/X_o (initial COD/initial MLSS) ratio in RUN 1 was only 0.11 mg COD/ mg MLSS, whereas this ratio was between 0.3-0.44 mg COD/mg MLSS in RUNS 2-5 which had higher initial COD values. Low substrate in RUN 1 should have resulted in death and lysis of microorganisms, which eventually led to higher SMP in this run.

In parallel bioregeneration reactors in RUNS 1-5 (Table 3.3), lower soluble COD concentrations were measured (15-20 mg/L) at the end of aeration. This was attributed to the adsorption of adsorbable organic matter onto the bioregenerated activated carbon. It is well known that the major portion of the residual organics in activated sludge systems consists of soluble microbial products (SMP) synthesized by the biomass (Barker and Stuckey, 1999) and a major part of the SMP is adsorbable on activated carbon and

nonbiodegradable (Schultz and Keinath, 1984). A great portion of SMPs has molecular weights greater than 1600 g/mole. They are concentrated on activated carbon and are not desorbed into the bulk solution (Schultz and Keinath, 1984). Therefore, in the present study, we should not expect bioregeneration of the adsorbed SMP. Phenol removal profiles in batch biodegradation reactors followed a linear trend with respect to time (Figures 4.7a-4.11a), indicating that a zero-order kinetic model was applicable (R^2=0.93-1) for biodegradation (Schultz and Keinath, 1984). The zero-order biodegradation rate constants for phenol removal were determined for the first 24-48 hours of aeration when substrate concentration was sufficiently high. These were calculated to be between 4.88 and 6.36 mg phenol/L.h, where the S_0/X_0 ratios (initial substrate to microorganism ratio in batch reactors) in RUNS 1-5 were between 0.11 to 0.17 mg phenol/ mg MLSS. In a previous literature study performed with phenol and 2,4-dichlorophenol, it was found that the removal probability of phenols by biosorption was very small and could be disregarded (Ha and Vinitnantharat, 2000). Also, removal of phenol due to volatilization was very low for the experimental conditions of our study, and it was therefore disregarded. A control experiment was performed in the absence of activated sludge to investigate the amount of phenol removal through abiotic means such as volatilization, sorption to plexiglass and photodegradation. This control experiment showed that during the first 24 hours of aeration only 0.6 mg/L phenol was removed, and after an aeration of 7 days only 9.6 mg/L phenol was removed by abiotic means in the 2 L reactor with an initial phenol concentration of 200 mg/L.

4.1.3.2. Investigation of Bioregeneration by Oxygen Uptake Rates. Phenol is known to be highly biodegradable in acclimated activated sludge cultures and activated carbon addition does not enhance biodegradation of phenol (Schultz and Keinath, 1984). However, the nonacclimated activated sludge in our study was inhibited at the start of each biodegradation and bioregeneration batch as shown by the initially very low oxygen uptake rates (OUR), which increased gradually during the first hours (Figures 4.7b-4.11b). Oxygen uptake rates were found to be higher in bioregeneration reactors compared with biodegradation reactors, particularly during the first 24 hours of aeration (Figures 4.7b-4.11b). This difference was statistically significant as determined by paired t-test at 95% confidence level. In bioregeneration reactors, desorbed phenol molecules were more easily biodegraded, since phenol concentration in the bulk liquid and hence inhibition was lower.

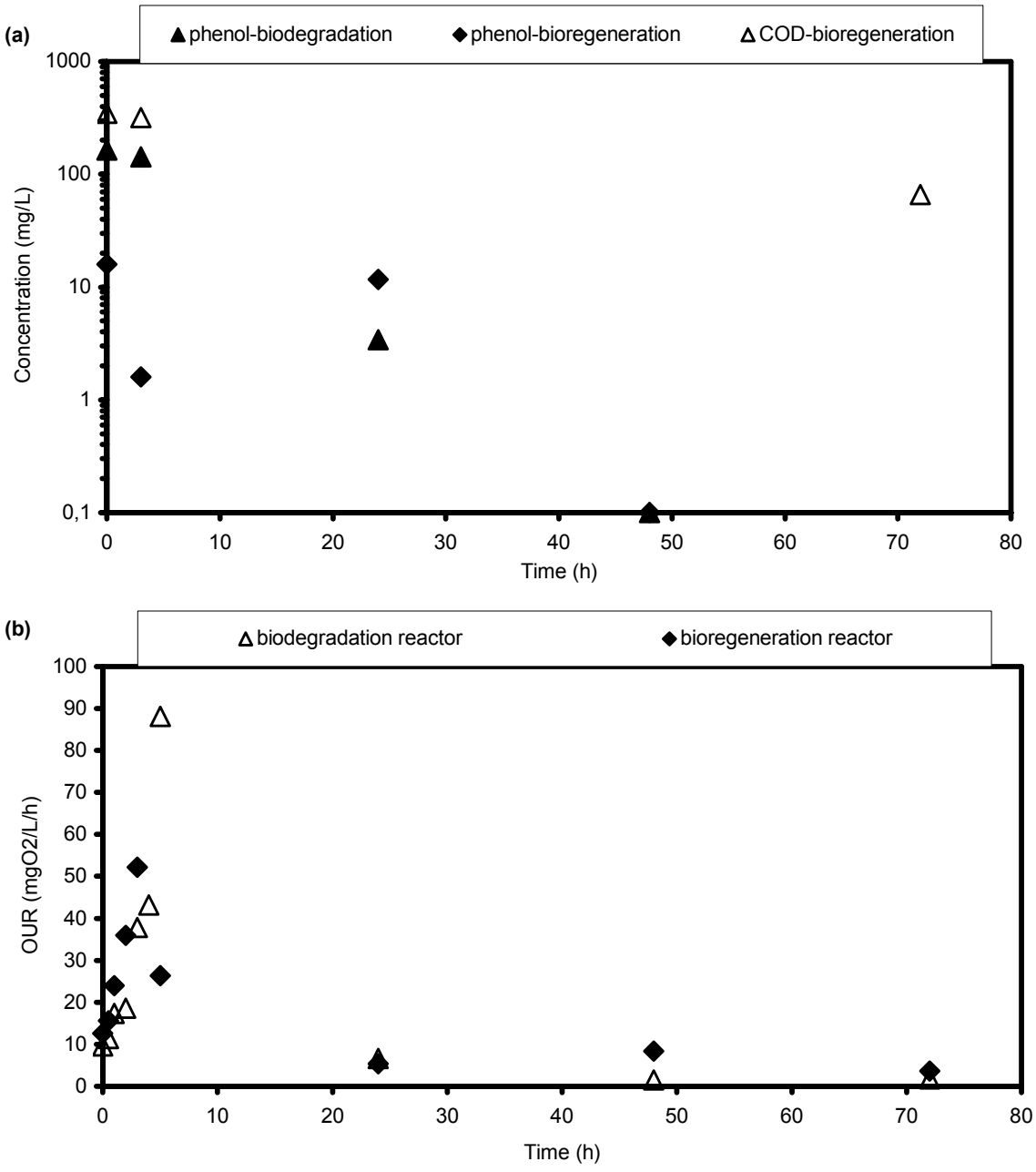


Figure 4.7. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 1000 mg/L SA4 (powdered, thermally activated) in RUN 1.

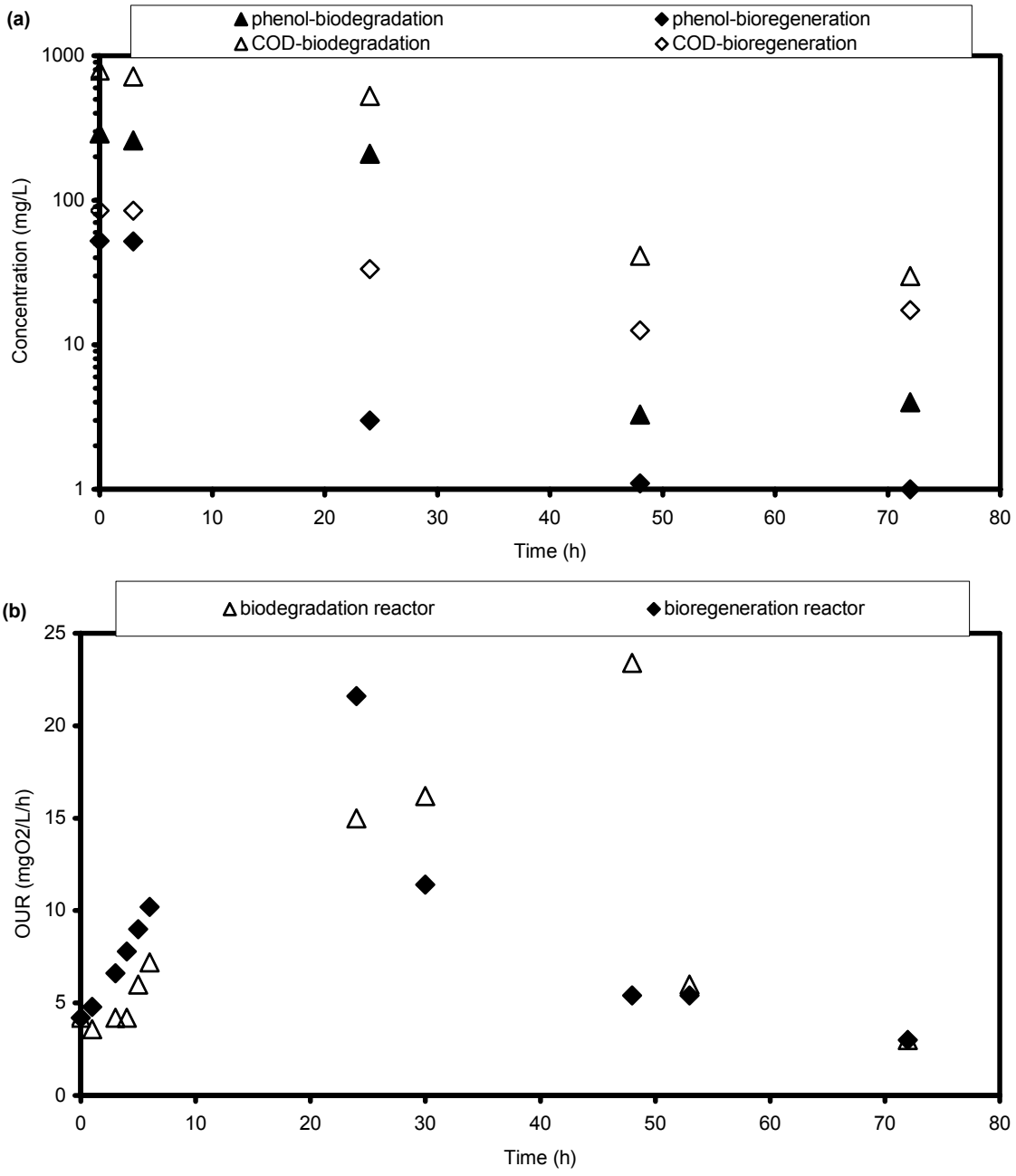


Figure 4.8. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L SA4 (powdered, thermally activated) in RUN 2

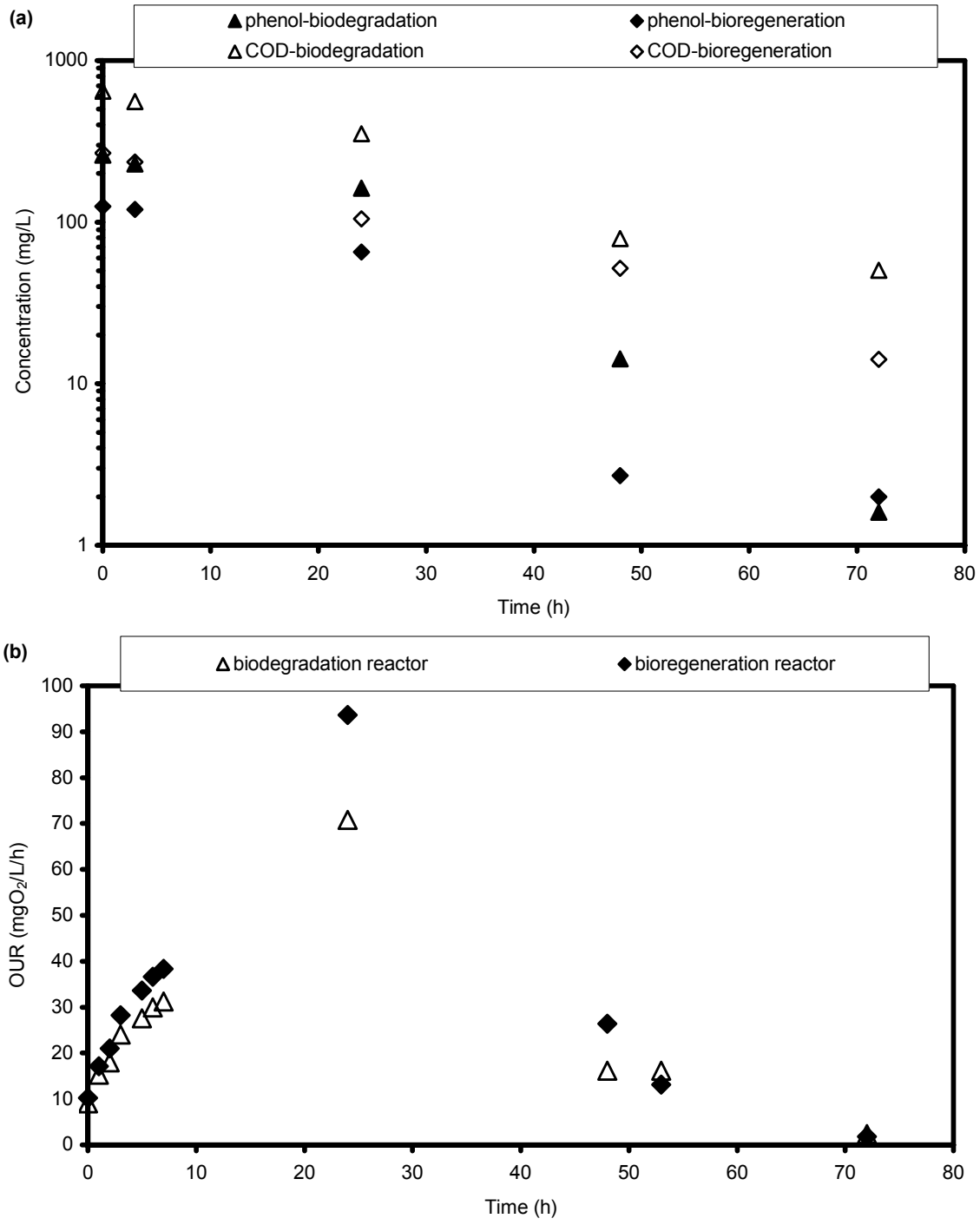


Figure 4.9. a) phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L CA1 (powdered, chemically activated) in RUN 3.

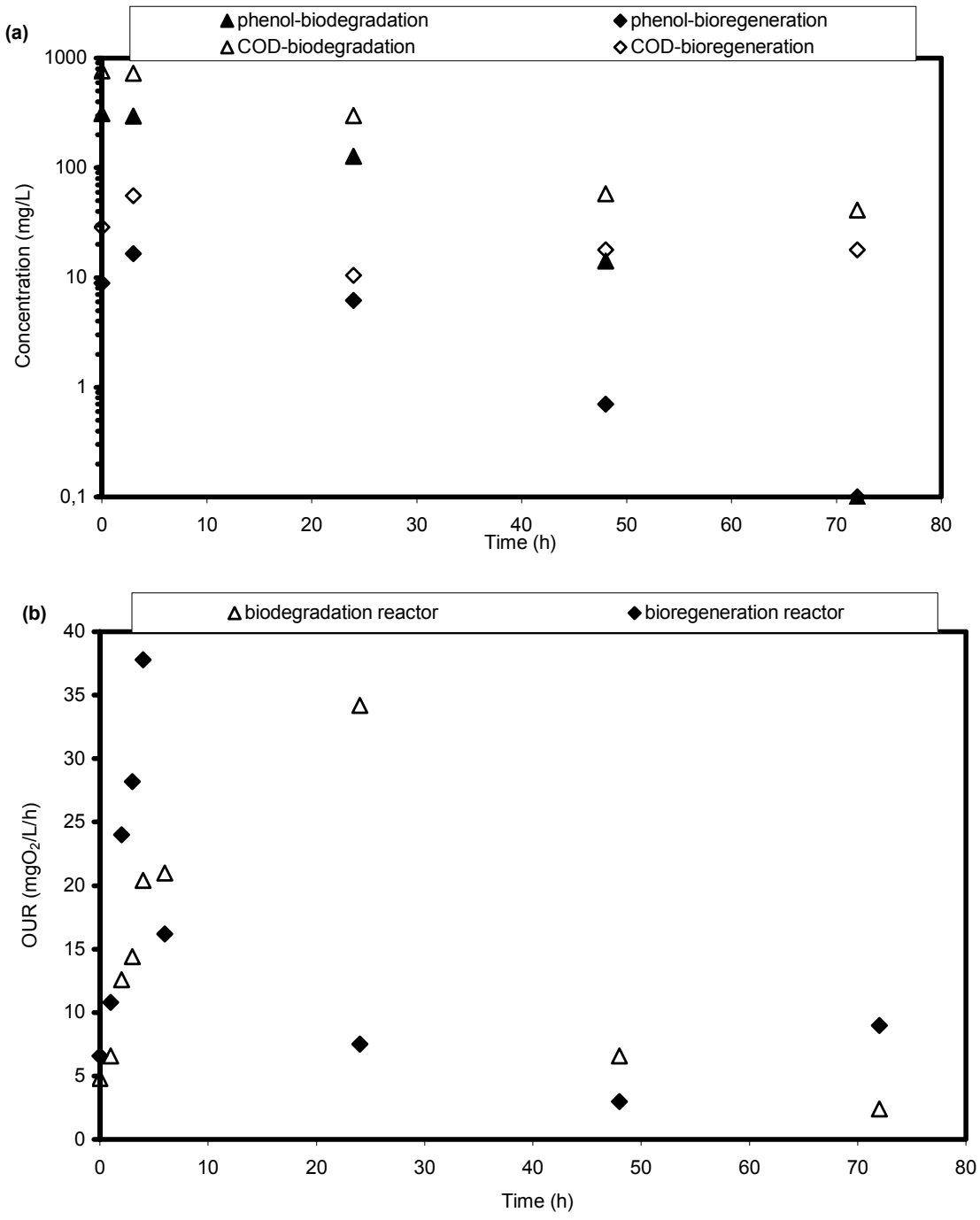


Figure 4.10. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L PKDA (granular, thermally activated) in RUN 4.

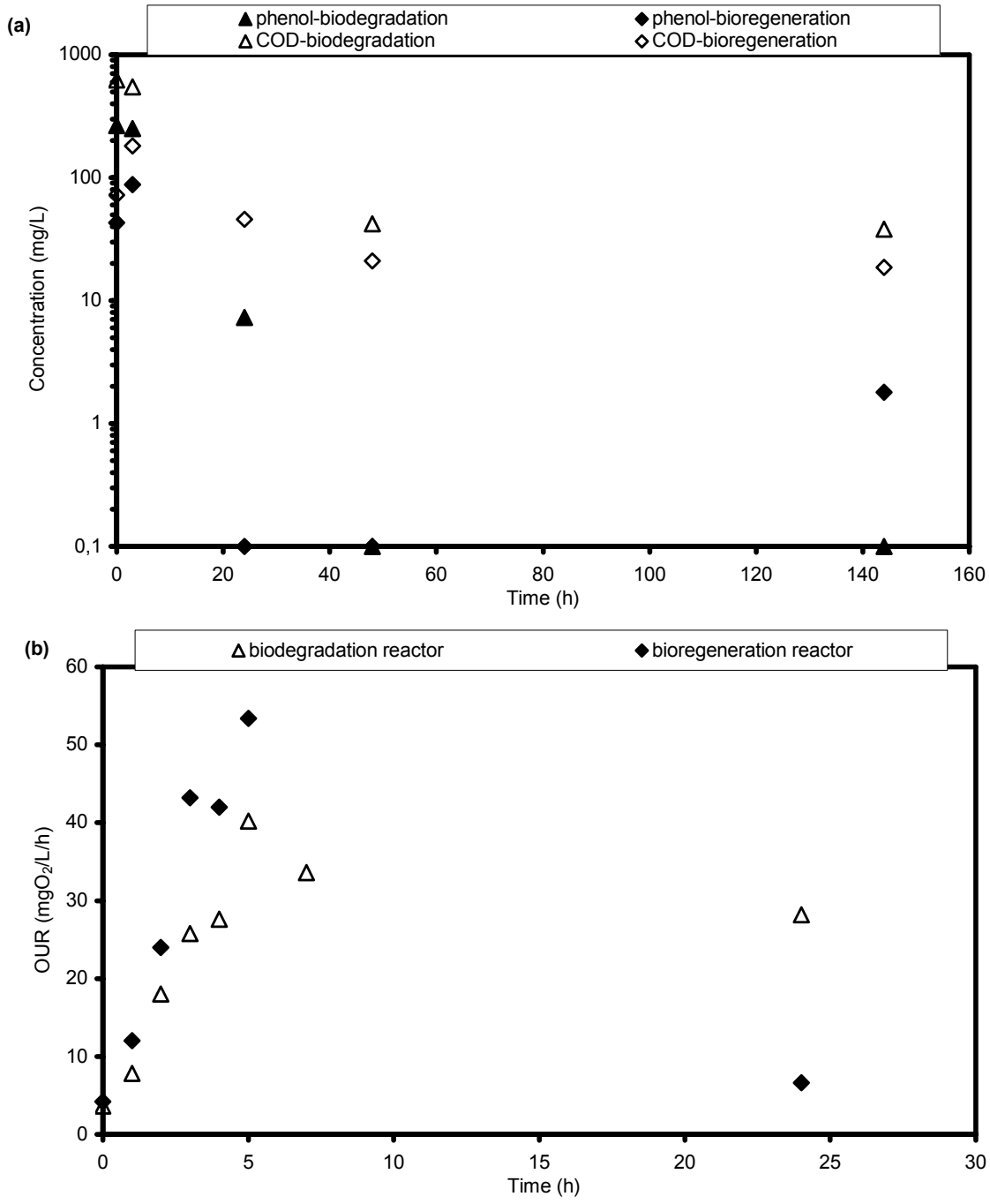


Figure 4.11. a) Phenol and COD profiles b) OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L CAgran (granular, chemically activated) in RUN 5.

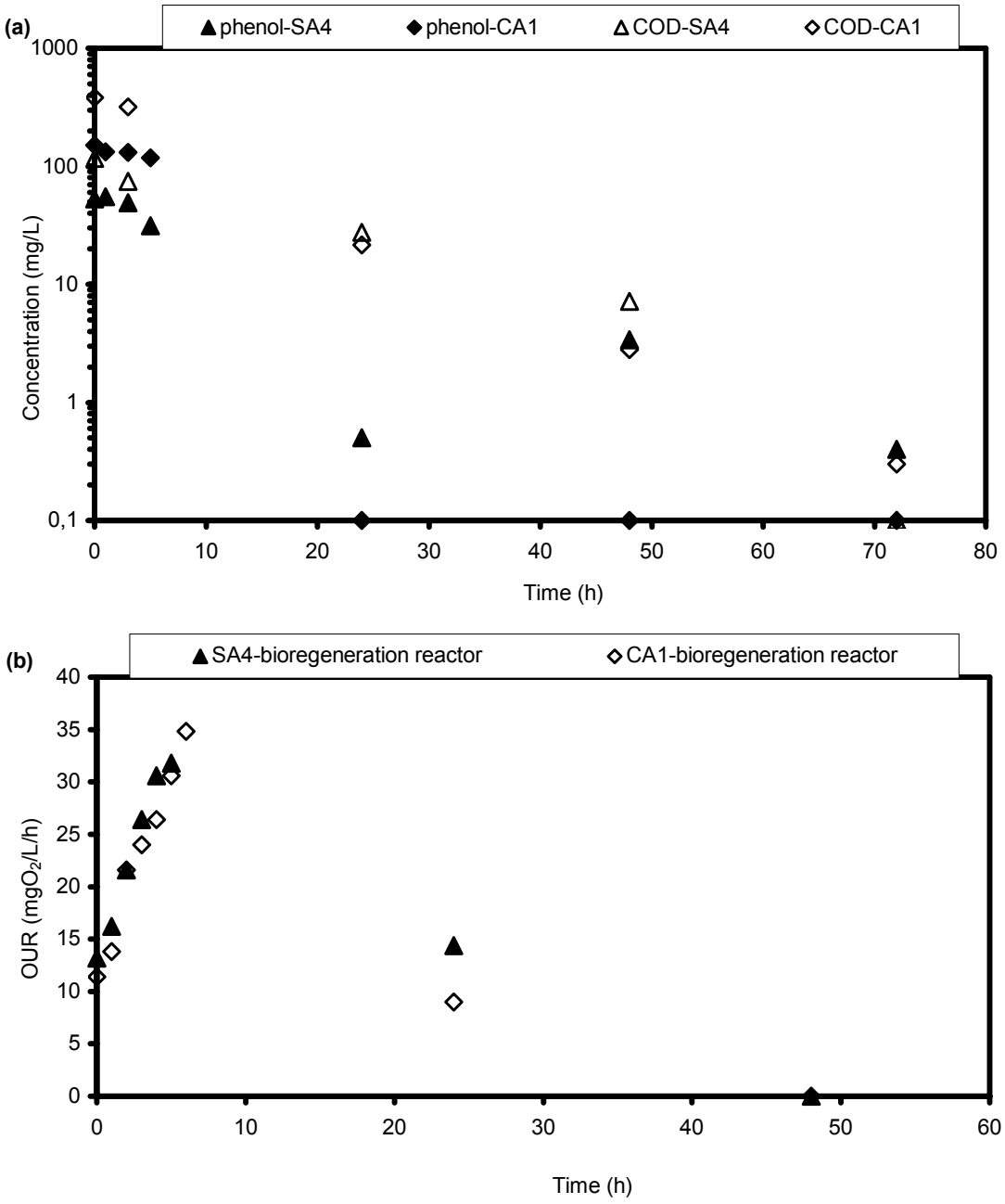


Figure 4.12. a) Phenol and COD profiles b) OUR profiles in bioregeneration reactors with 2000 mg/L SA4 (powdered, thermally activated) and CA1 (powdered, chemically activated) in RUN 6.

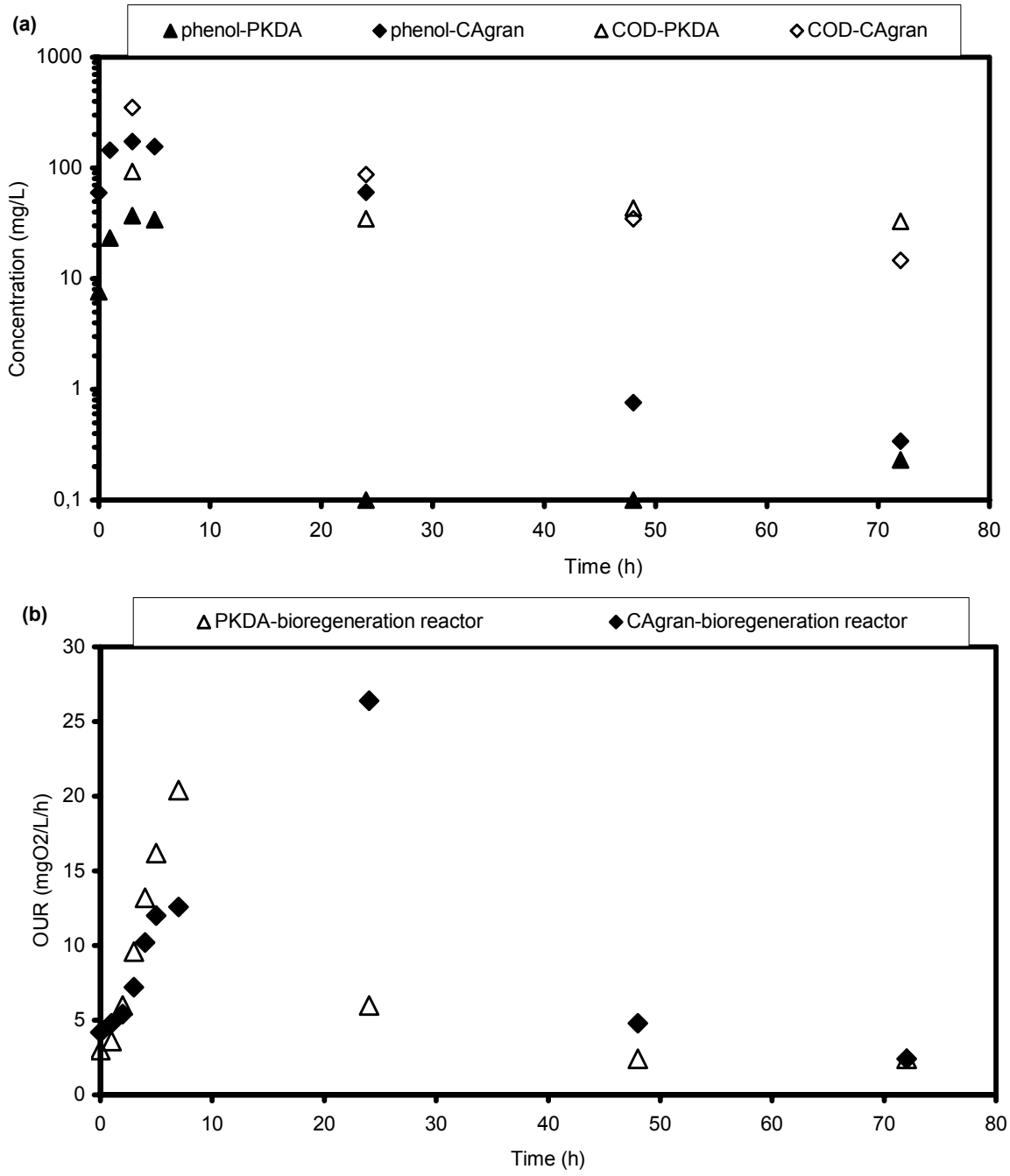


Figure 4.13. a) Phenol and COD profiles b) OUR profiles in bioregeneration reactors with 2000 mg/L PKDA (granular, thermally activated) and CAgran (granular, chemically activated) in RUN 7.

Interaction of oxygen with the carbon surface, which is considered to be an oxygen consuming process in literature (Den Blanken, 1983), might have also contributed to the higher OUR values in bioregeneration reactors. This was particularly observed in the case of thermally activated carbons SA4 and PKDA. Control OUR experiments were performed with virgin activated carbons in the absence of phenol and microorganisms. These experiments showed that virgin SA4 and PKDA showed OUR values of about 1.8 mg/L.h during the first hour of experiment and OUR values decreased gradually down to zero at the end of 24 hours. Hence, in the bioregeneration reactors with SA4 and PKDA, higher OUR values were also partly related to the oxygen uptake of carbon, particularly during the first few hours of aeration. However, in the case of chemically activated carbons CA1 and CAgran, during the first hour of control experiment negative OUR values of about -1.5 mg/L.h were measured. Chemically activated carbons seemed to release oxygen during the first hour of experiment instead of consuming oxygen, probably by physical means due to stirring during the measurement of OURs. This showed that the surface of thermally activated carbons was more reactive towards oxygen compared with chemically activated carbons. However, in the control experiment, after the first hour chemically activated carbons also consumed oxygen with OUR values of less than 1.2 mg/L.h that decreased gradually down to zero at the end of 24 hours. Another literature study (Jonge et al., 1996b) also demonstrated that thermally activated carbon SA4 was more reactive towards oxygen compared with chemically activated CA1.

In another study (Vidic et al., 1993), oxygen uptake of virgin carbon was found to be very low so that it could be disregarded. However, in the same study, the amount of oxygen consumed during the adsorption of phenolic compounds was linearly proportional to the amount of irreversibly adsorbed compound. This oxygen consumption was due to oxidative polymerization of phenolic substances during adsorption on activated carbon. Hence, the higher OUR values in the bioregeneration reactors of our study might be affected also by the oxidative coupling reactions, which consume oxygen. In another literature study, another hypothesis was that partial chemical oxidation of phenol to hydroquinone caused oxygen consumption in the absence of microorganisms (Den Blanken, 1983).

In bioregeneration RUNS 6 and 7 (Table 3.3) performed with the SA4-CA1 couple (Figure 4.12) and the PKDA-CAgran couple (Figure 4.13), OUR values in parallel reactors were comparable during the first 5-7 hours of aeration. OUR values were also statistically similar as determined by paired t-test at 95% confidence level. OUR values in reactors containing thermally activated carbons (SA4 and PKDA) were only slightly higher than the ones containing chemically activated carbons (CA1 and CAgran) during this period (Figures 4.12b, 4.13b). However, particularly during the first hours of aeration, the bulk liquid phenol concentrations in the parallel reactors were much higher in the bioregeneration reactors containing chemically activated carbons due to higher desorbability from these carbons (Figures 4.12a, 4.13a). It can be said that in reactors containing SA4 or PKDA, bulk phenol concentrations as low as 20-50 mg/L resulted in a comparable inhibition as in reactors containing CA1 and CAgran which had bulk phenol concentrations as high as 120-180 mg/L. Thus, it can be proposed that the phenol loading on thermally activated carbons also contributed to inhibition of exoenzymatic reactions in addition to the inhibition caused by bulk phenol. Therefore, exoenzymatic activity seemed to be an important factor in phenol biodegradation and bioregeneration.

4.1.3.3. Quantification of Bioregeneration. As done in a literature study (Vinitnantharat et al., 2001), bioregeneration efficiencies were estimated using the Freundlich adsorption isotherm constants and the phenol concentrations in the bioregeneration reactors (Table 4.5). Using the Freundlich parameters, K and 1/n, previously obtained from the adsorption isotherms, the amount of phenolic substance adsorbed before the bioregeneration step was calculated by the following equation:

$$Q_1=(C_1-C_e) * V/W=KC_e^{1/n} \quad (4.6)$$

where,

Q_1= the amount of a phenolic compound adsorbed before the bioregeneration step per unit weight of carbon, mg/g

C_1= initial concentration of the phenolic compound, mg/L

C_e= equilibrium concentration, mg/L

V= total volume of sample, L

W= weight of activated carbon, g

After bioregeneration, the amount of adsorbed substrate left on activated carbon (Q′) was determined by Equation 4.7:

$$Q' = K\, C_e'^{\,1/n} \quad (4.7)$$

where,

C_e' = the adsorbate concentration in the bulk liquid.

After addition of the substrate and equilibration, the additional amount of substrate adsorbed (ΔQ_2) was calculated by Equation 4.8:

$$\Delta Q_2 = (C_2 - C_{2e}) * V/W \quad (4.8)$$

where,

C_2 = the concentration at the beginning of equilibration

C_{2e}= the equilibrium concentration.

Hence total adsorbability (Q_2) was calculated as follows:

$$Q_2 = Q' + \Delta Q_2 = K\, C_{2e}^{\,1/n} \quad (4.9)$$

Then, the quantity of bioregenerated phenol was calculated as follows:

$$\text{Quantity bioregenerated (mg/g)} = Q_1 - Q' = Q_1 - (Q_2 - \Delta Q_2) \quad (4.10)$$

$$\text{Percentage of bioregeneration (\%)} = 100 * (Q_1 - Q')/Q_1 \quad (4.11)$$

Table 4.5. Bioregeneration rates and efficiencies for activated carbons loaded with phenol.

Carbon type	Physical form	Activation type	Initial MLSS (mg/L)	Initial phenol loading (mg /g)	Bioregen. rate constant k (h^{-1})	Bioregen. Efficiency (%)	Reversibility of adsorption (%)
SA4	powdered	thermal	2325	161.4	0.0120	58.1	20.3
CA1	powdered	chemical	2295	137.5	0.0312	93.6	86.6
PKDA	granular	thermal	1865	170.0	0.0126	66.6	25.8
CAgran	granular	chemical	1400	140.3	0.0206	84.8	87.5

In literature, it was assumed that bioregeneration kinetics followed a first-order pattern (Kim et al., 1997; Vinitnantharat et al., 2001). Considering that bioregeneration is basically biodegradation following desorption, the bioregeneration rate should be related to the amount of previously adsorbed phenol rather than the bulk phenol concentration.

Therefore, although phenol removal in biodegradation reactors followed a zero-order pattern, bioregeneration kinetics followed a first-order pattern as evidenced from high correlations (R^2=0.91-1). First-order bioregeneration rate constants for each carbon type (Table 4.5) were calculated as follows;

$$dQ'/dt = -k\,Q' \quad (4.12)$$

$$\ln Q'/Q_1 = -k\,t \quad (4.13)$$

where

Q_1 = the amount of adsorbed substrate at the beginning of bioregeneration, mg/g

Q' = the amount of adsorbed substrate left at time t during bioregeneration, mg/g

k = first-order bioregeneration rate constant, h^{-1}

The rate of bioregeneration at any time was proportional to the amount of adsorbate left on activated carbon at that particular time. Hence, renewal of active sites occurred faster at the beginning and declined gradually. First-order bioregeneration rate constants were higher for chemically activated carbons CA1 and CAgran compared with thermally activated carbons as expected. High correlations (R^2=0.93-0.99) showed that the first-order kinetic model could also be applied to phenol removal in biodegradation reactors when the total aeration time of 72 hours was considered. First-order phenol biodegradation rate constants, which were calculated for comparison with bioregeneration rate constants over the same time period, were determined to be between 0.06 and 0.07 h^{-1}. Bioregeneration rate constants between 0.012 and 0.0312 h^{-1} (Table 4.5) were much lower than the first-order biodegradation rate constants due to diffusion through the carbons. In the case of thermally activated carbons it was also due to high irreversible adsorption. Desorption was obviously a rate limiting factor for bioregeneration.

4.1.3.4. Bioregeneration due to Concentration Gradient. Previous desorption equilibrium studies had shown that desorption rate was higher in the case of powdered activated carbons compared with their granular countertypes due to higher diffusivity. Thereby, it can be deduced that diffusivity would also control bioregeneration rates, particularly in the first few hours of aeration. However, the bioregeneration rates in this study did not depend on the particle size and porous structure of the carbon when the total aeration period of

several days was considered. The highly macroporous structure of GAC particles (Table 3.1) did not result in higher bioregeneration rates or bioregeneration efficiencies. This is in contrast to the theory that the porous structure of the activated carbon is a factor that determines to a great extent both the rate and degree of biological regeneration (Klimenko et al., 2002). The reason for this finding may be that the macropores of activated carbons were not mainly responsible for adsorption of phenol. Hence, desorption did not mainly occur from macropores, but from micropores and mesopores which have comparable volumes for each PAC and GAC countertype (Table 3.1).

Bioregeneration of chemically activated carbons was higher in accordance with their higher reversibility of adsorption (Table 4.5). Also, post-bioregeneration adsorption experiments (Section 3.3.3) made direct measurement of bioregeneration possible by using adsorption capacities before and after bioregeneration (Eq. 2.7 in Section 2.3.6.2). These direct measurements of bioregeneration also resulted in bioregeneration percentages as high as 83% for the chemically activated carbons. It is a fact that bioregeneration is controlled by the reversibility of adsorption. Figure 4.14a shows that a great portion of the initial phenol loading on chemically activated carbons was removed at the end of bioregeneration. Also a considerable amount of phenol loaded on thermally activated carbons was removed by bioregeneration. Post-bioregeneration adsorption studies in RUN 6 showed that about 55 % and 42 % of bioregeneration occurred during the first 5 hours for SA4 and CA1, respectively. Figure 4.14b shows the desorption profiles from loaded activated carbons in the absence of microorganisms. These were obtained from the previous desorption experiments. It is obvious that in the absence of microorganisms very little desorption occurred from loaded carbons (Figure 4.14b) compared with the bioregeneration reactors in RUNS 2-7 (Figure 4.14a). Thus, it can be stated that desorption serves as a prerequisite step for bioregeneration, but desorption proceeds as long as the bulk phenol is reduced by biological activity and a concentration gradient is created between the carbon surface and the bulk liquid.

4.1.3.5. Bioregeneration due to Exoenzymatic Reactions. In the case of thermally activated carbons bioregeneration efficiencies were much higher than their total desorbabilities (Table 4.5). Direct measurement of bioregeneration via post-bioregeneration adsorption experiments also resulted in bioregeneration efficiencies up to 60%, which also confirmed

the efficiencies in Table 4.5 obtained by the Freundlich analogy. This indicated that some exoenzymatic reactions might have occurred so that carbon bioregeneration was more than expected. In literature, some researchers stated that bioregeneration involved extracellular enzymes (Perrotti and Rodman, 1974; Kim et al., 1997; Sirotkin et al., 2001). Others proposed a mechanism involving desorption due to a concentration gradient only and stated that non-desorbable compounds could not be bioregenerated (Schultz and Keinath, 1984; Speitel et al., 1989; Jonge et al., 1996a). It should be noted that there was still non-desorbed phenol on activated carbon at the end of bioregeneration batches in RUNS 2-7 (Figure 4.14a), whereas all of the phenol was removed in biodegradation reactors (Figures 4.7a-4.11a). Hence, if the bioregeneration theory including exoenzymes is valid in every case, one should expect that all of the phenol loading on the activated carbon would be biodegraded in the bioregeneration reactors. However, it can be speculated that exoenzymes are not small enough to reach narrow micropores, but they can react with the adsorbed phenol on the meso- and macro pores of thermally activated carbons (SA4 and PKDA) which are prone to chemisorption and oxidative polymerization of phenol (Jonge et al., 1996b).

In literature, it is stated that for an enzyme to actively catalyze a reaction in a pore, the pore diameter should be at least three times greater than the enzyme size to allow the enzyme to approach and induce a fit (Xiaojian et al., 1991). Considering that the average molecular diameter of a smallest monomeric enzyme is about 31-44 Å, it was stated that the diameter of the pore in which the exoenzyme can catalyze should be larger than 10 nm (Xiaojian et al., 1991) which does not include the micropores (Φ<2nm) and some of the mesopores (Φ 2-50nm). Since phenol is mainly adsorbed in pores with a diameter under 0.7 nm (Martin et al., 2002), the effect of hypothetical exoenzymatic reactions is expected to be limited. There are other researchers who also state that the micropores of the activated carbon are occupied by adsorbed molecules and are not subjected to bioregeneration (Klimenko et al., 2002). Some researchers support the idea that inaccessibility of micropores for exoenzymes does not contradict the probable exoenzymatic activity in meso- and macropores (Sirotkin et al., 2001; Klimenko et al., 2003). However, others (Xiaojian et al., 1991) state that no evidence exists that exoenzymes are involved in ring hydroxylation and ring fission reactions of aromatic

compounds and phenols, and propose that bioregeneration hypothesis including exoenzymatic reactions is not valid for these compounds.

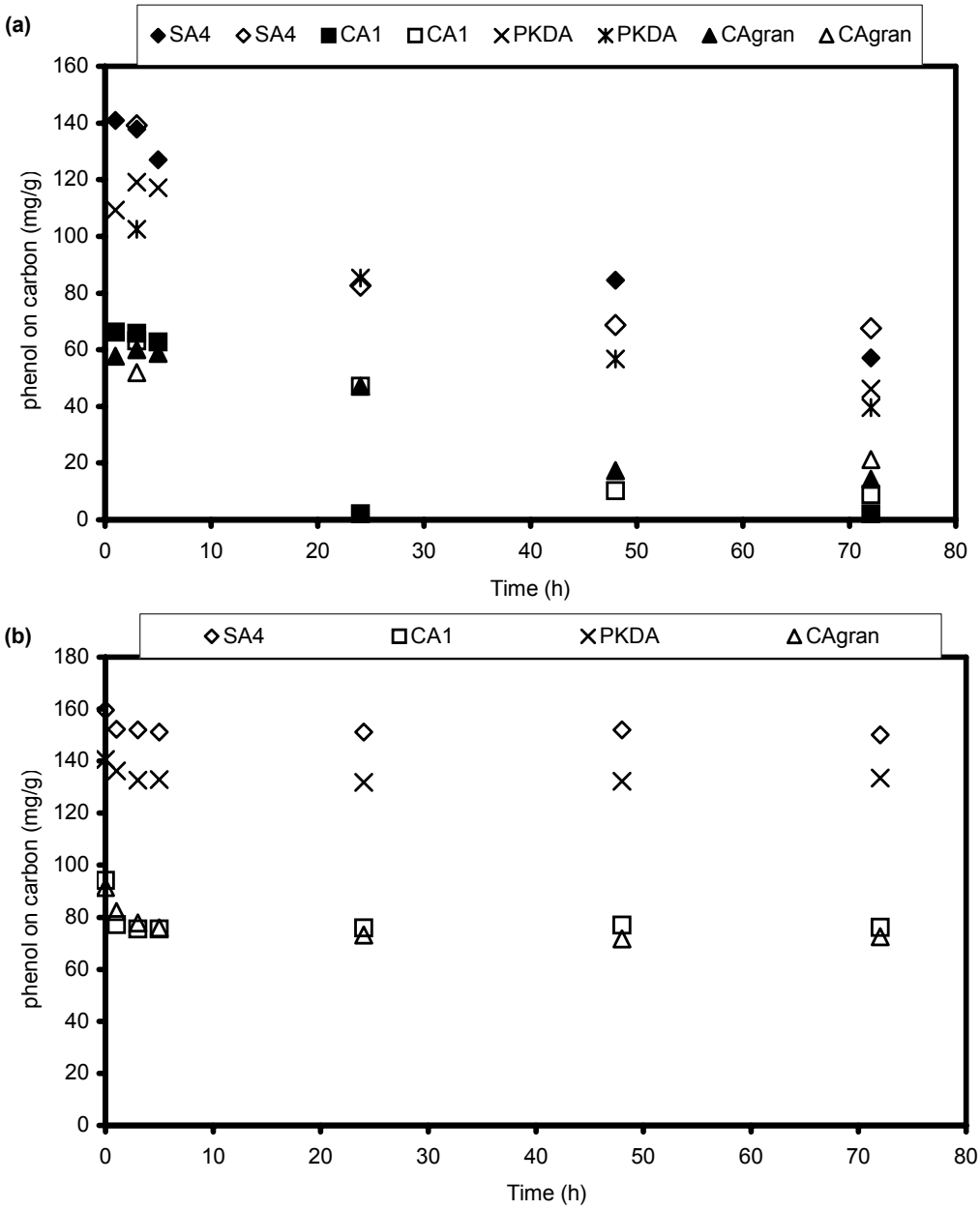


Figure 4.14. Profiles of phenol loadings on activated carbons SA4 (powdered, thermally activated), CA1 (powdered, chemically activated), PKDA (granular, thermally activated) and CAgran (granular, chemically activated) a) in bioregeneration reactors in RUNS 2-7 and b) during desorption equilibrium.

According to the theory including exoenzymes, some enzymes excreted by the bacteria could easily diffuse into the pores and react with the adsorbed substrates. Then, hydrolytic decay of the substrate may occur or further desorption may take place due to the weak adsorbability of organic matter reacting with the enzyme. In this present study, in the case of thermally activated carbons some of the chemically adsorbed and strongly bound phenol molecules were probably not desorbed even after reaction with the enzyme. For chemically activated carbons (CA1 and CAgran), exoenzymes were not effective on bioregeneration. The reason for this might be that adsorption type was physical and was already highly reversible for these carbons as discussed earlier, and the non-desorbed phenol was probably present in the micropores where enzymes could not reach.

4.1.3.6. Oxidative Coupling of Phenol. Considering the aerobic biological treatment of phenols in the presence of oxygen, oxidative coupling of phenols on activated carbon is also very important for bioregeneration. Phenol molecules undergoing an oxidative coupling reaction may be irreversibly adsorbed on activated carbon (Vidic et al., 1993), which in turn may result in a low bioregeneration efficiency. Phenoxy radicals, formed by the removal of a hydrogen atom from each phenolic molecule, can participate in direct coupling with other phenoxy radicals at room temperature, activated carbon surface serving as a catalyst (Vidic et al., 1993). Another study (Jonge et al., 1996b) demonstrated that adsorption of a phenolic compound, o-cresol, was increased in the presence of molecular oxygen due to oxidative polymerization, where adsorption of a nonphenolic compound, 3-chlorobenzoic acid, was not affected by the presence of oxygen. Oxidative coupling of phenolic compounds, except nitrophenols, was found to decrease the reversibility of adsorption (Vidic et al., 1993). In the present study, irreversible adsorption was higher and bioregeneration was lower in the case of thermally activated carbons. This was explained by the affinity of thermally activated carbons towards oxygen and changes in surface chemistry upon contact with oxygen (Jonge et al., 1996b). Thermal activation is carried out in the absence of oxygen and leads to a more reactive surface. Contrary to this, chemically activated carbons have a surface with fully oxidized active sites so that interaction with oxygen does not affect the surface.

4.1.3.7. ESEM Analyses of Carbon Surfaces. Environmental Scanning Electron Microscopy (ESEM) analyses were used to investigate the attachment of microorganisms on activated carbons. ESEM analyses help to determine if the microorganisms attach on the inner side or outer surface of activated carbon, which is of crucial importance for bioregeneration (Ha et al., 2000). It is expected that microorganisms attached to the inner side of activated carbon can significantly contribute to the assimilation of adsorbed compounds. It is known that microorganisms have the tendency to attach to sites near the substrate for efficient uptake (Weber et al., 1978). This microbial activity enhances bioregeneration by inducing successive desorption of adsorbed substrate (Ha et al., 2000). The ESEM analyses with powdered carbons SA4 and CA1 showed that microorganisms were attached both on external surface and interval cavities of PAC particles (Figure 4.15). The attached microorganisms might have contributed to the bioregeneration of activated carbon either by creating a concentration gradient between the carbon surface and the bulk or by excreting extracellular enzymes through the activated carbon pores.

The external surface of carbons contained mainly protozoa and filamentous, long rod- and spiral-shaped bacteria (Figure 4.15). Groups of short rod and cocci-shaped bacteria were found to be attached only in internal cavities of carbon particles, probably due to turbulent fluid dynamics on the external surface. The holes, cravasses and ridges on activated carbon surface are considered to be sites where microorganisms are shielded from shear forces providing a favorable environment for growth (Weber et al., 1978). The bacteria colonizing on the carbon surface produce a nonhomegenously distributed polysaccharide slime matrix over the carbon surface (Weber et al., 1978). Also in the present study, a slime matrix forming a gelatinous structure was clearly observed as shown in Figure 4.16. The slime matrix consisting of cellular debris and extracellular polymeric substances, prevented the monitoring of individual microorganisms on most of the carbon surfaces (Figure 4.16). Within the concept of this work, there was no evidence that this slime matrix prevented desorption and consequently bioregeneration of the loaded phenol molecules.

The basic findings obtained in Section 4.1.3 have been published as a paper (Aktaş and Çeçen, 2006b).

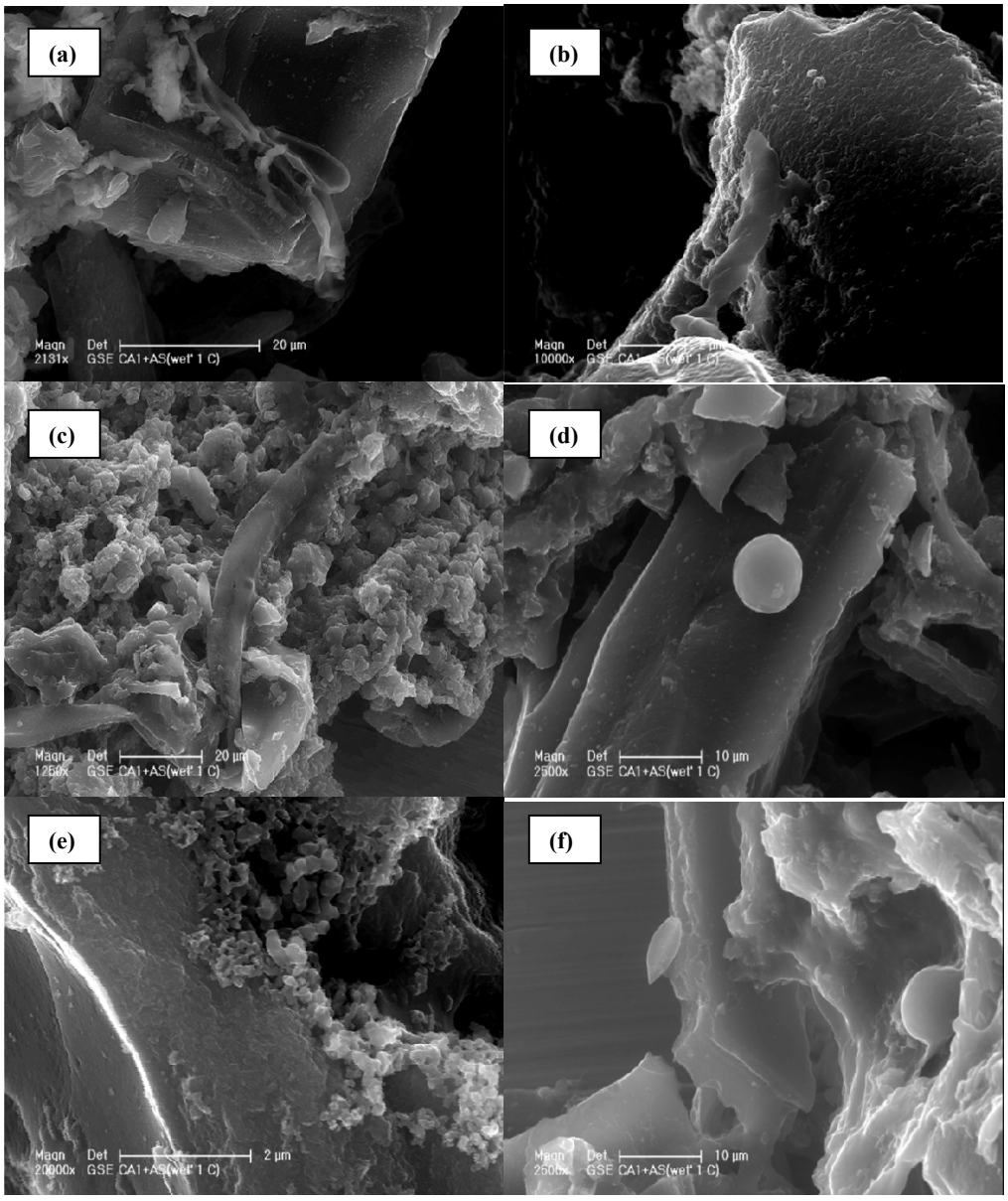


Figure 4.15. Scanning Electron Micrographs of microorganisms on activated carbon a) filamentous bacteria b) spiral-shaped bacteria c) a protozoan d) a spherical protozoan attached to the carbon surface e) group of cocci-shaped bacteria clustered in the carbon cavities f) a stalked protozoan attached to carbon cavities.

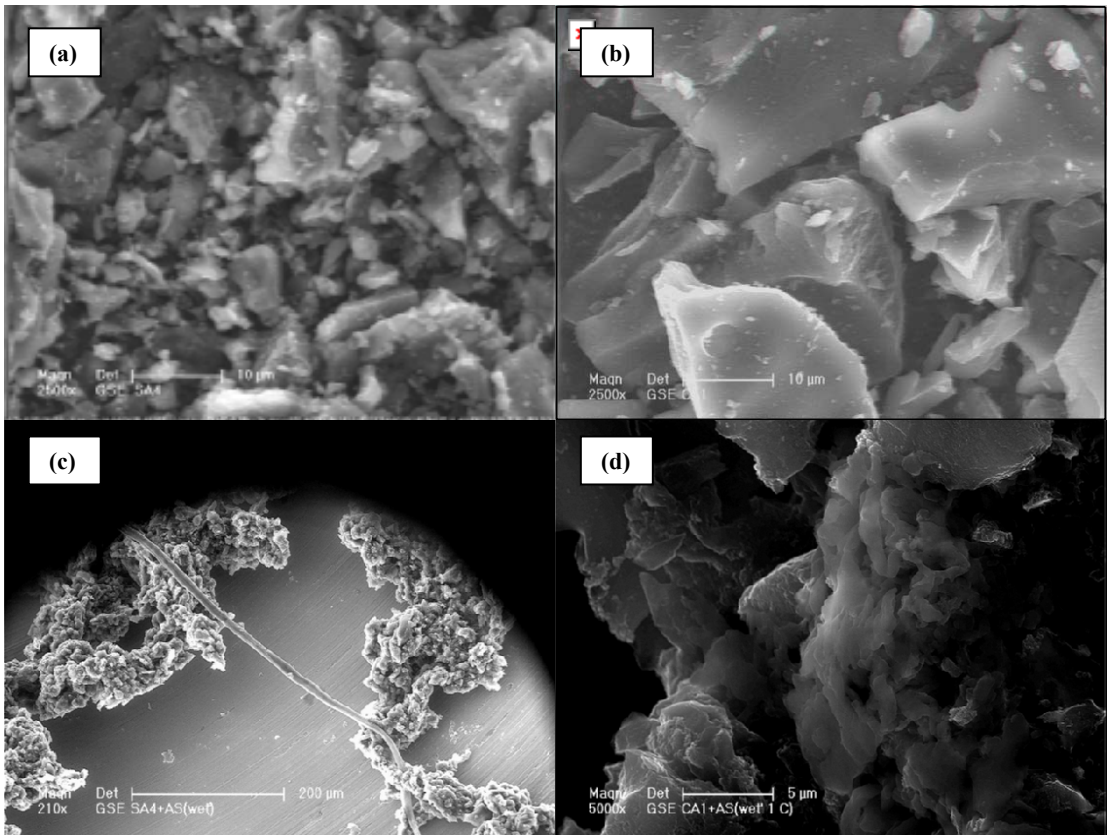


Figure 4.16. Scanning Electron Micrographs of a) virgin dry SA4 b) virgin dry CA1 c) slime matrix covering parts of carbon surface forming a bright white gelatinous structure.d) Slime matrix covering microorganisms on activated carbon

4.2. Experiments with 2-Chlorophenol as a Model Compound

In the second part of experimental work, 2-chlorophenol was used as the model compound because it is known to be adsorbable on activated carbon, but less biodegradable than phenol. Difference in adsorbabilities, desorbabilities and biodegadabilities of two aromatic compounds will enable us to investigate the effect of the target aromatic compound on bioregenerability of different activated carbon types.

4.2.1. Adsorption Studies with 2-Chlorophenol

The equilibrium time for adsorption studies was determined as 7 days for SA4 and PKDA, and 1 day for CA1 and CAgran as seen in Figure 4.17. The adsorption isotherm data for 2-chlorophenol are plotted in Figure 4.18. The adsorption isotherm data fitted the Freundlich equation (Eq. 4.1), as in the previous adsorption isotherms with phenol. Freundlich adsorption isotherm constants obtained by regression analysis for 2-chlorophenol are shown in Table 4.6.

Table 4.6. Freundlich isotherm constants for 2-chlorophenol adsorption.

Carbon type	**Physical form**	**Activation method**	**K [(mg/g) (L/mg)$^{1/n}$]**	**1/n**	**R^2**
Norit SA4	Powdered	Thermal	86.5	0.1947	0.96
Norit CA1	Powdered	Chemical	13.6	0.4064	0.97
Norit PKDA	Granular	Thermal	60.4	0.2265	0.93
Norit CAgran	Granular	Chemical	8.2	0.5526	0.98

The K value, which is an indicator of adsorption capacity, was found to be higher for the thermally activated carbons compared with the chemically activated carbons (Table 4.6). The thermally activated carbons were obviously better adsorbers for 2-chlorophenol as in the case of phenol. Smaller K values obtained for the chemically activated carbons reveal that these carbons are not very suitable for adsorption of 2-chlorophenol. Lower 1/n values for the thermally activated carbons indicate that an increase in the activated carbon dose was more effective for thermally activated carbons rather than chemically activated

carbons. This can be seen more clearly in Figure 4.19, which illustrates the effect of carbon dose on equilibrium bulk 2-chlorophenol concentrations.

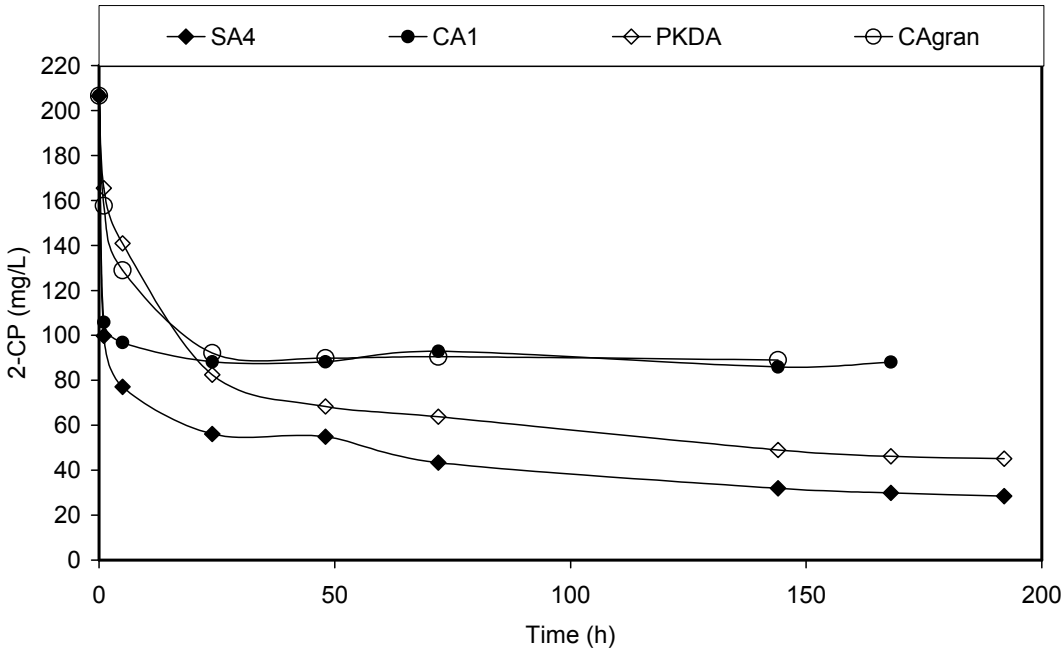


Figure 4.17. 2-Chlorophenol profiles during adsorption at an initial 2-CP concentration of 200 mg/L and 1 g/L of activated carbons SA4, CA1, PKDA and CAgran.

Previous adsorption studies with phenol had indicated high differences between the thermally activated carbons and chemically activated ones in terms of adsorption efficiency (Figure 4.2). In the studies with 2-chlorophenol, this difference also existed but was smaller (Figure 4.19). For all carbon types, 2-chlorophenol was better adsorbed than phenol. This difference was higher in the case of chemically activated carbons (Figures 4.2 and 4.19).

The difference in adsorption capacities between thermally and chemically activated carbons was certainly caused by their activation method, which eventually results in different surface characteristics. Equilibrium pH values obtained in 2-CP adsorption isotherm studies pointed out that the surface characteristics of the two thermally activated carbons SA4 and PKDA (pH between 7.1-8.4 depending on the carbon dose) were basic, and the surface characteristics of the two chemically activated (with phosphoric acid) carbons CA1 (pH between 3.9-5.5) and CAgran (pH between 5.4-6.9) were acidic as in the case of phenol. The inverse relationship between adsorption capacity and surface acidity was previously investigated in Section 4.1.1.

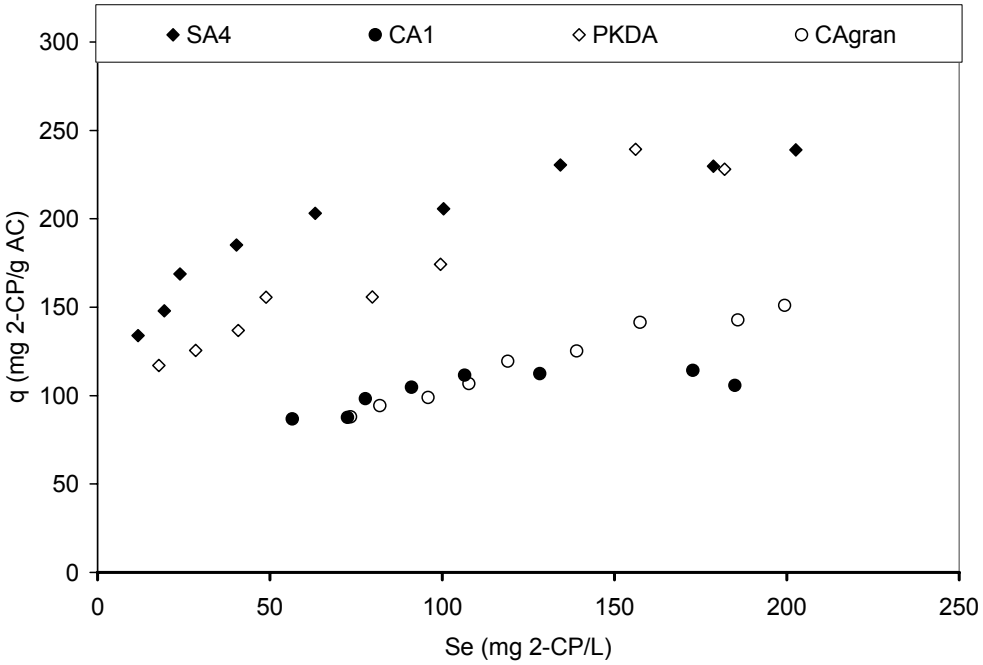


Figure 4.18. 2-Chlorophenol adsorption isotherms for each activated carbon type SA4 (powdered, thermally activated), CA1 (powdered, chemically activated), PKDA (granular, thermally activated) and CAgran (granular, chemically activated).

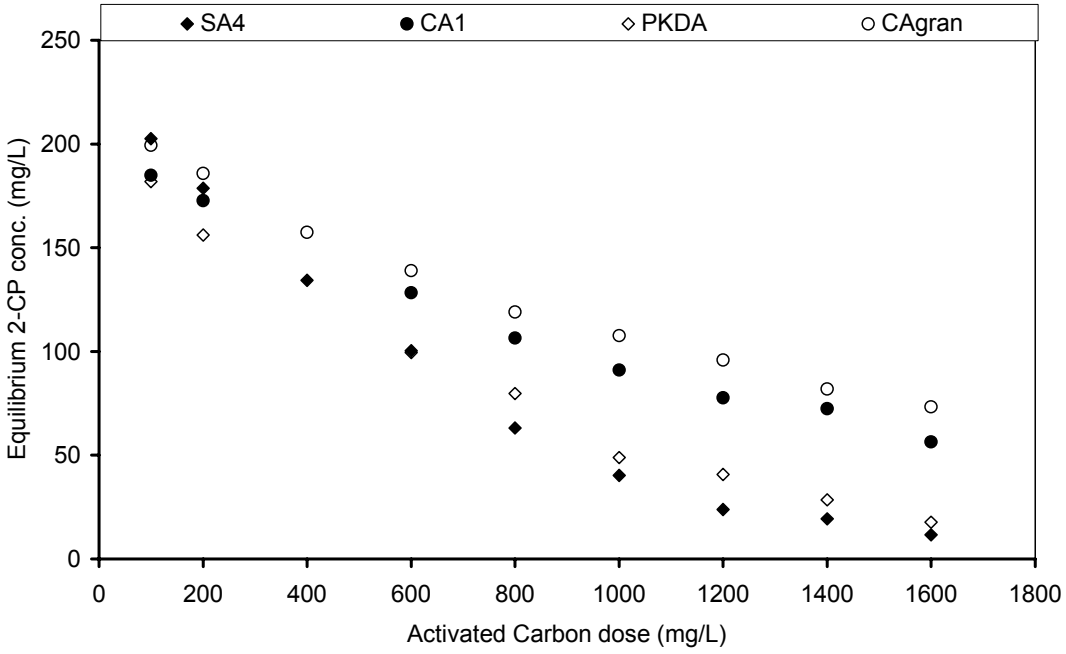


Figure 4.19. Effect of activated carbon dose on equilibrium 2-chlorophenol concentrations with an initial 2-chlorophenol concentration of 200 mg/L for each activated carbon type.

Figure 4.19 shows that the powdered and granular countertypes (SA4-PKDA and CA1-CAgran) do not significantly differ in terms of 2-chlorophenol adsorption, although powdered carbons exhibited slightly better adsorption. Considering that the granular activated carbons have higher macropore volumes than their powdered countertypes (Table 3.1), similar adsorption characteristics for both powdered-granular countertypes shows that 2-chlorophenol adsorption on macropores was much less significant compared with meso- and micropores as in the case of phenol. During the first few hours of adsorption (Figure 4.17) before reaching adsorption equilibrium, the powdered activated carbons (SA4 and CA1) adsorbed more 2-CP compared with their granular countertypes (PKDA and CAgran). Adsorption was faster onto PACs, probably due to higher intraparticle diffusivity through PAC compared with GAC, which has a higher diameter.

The isotherm curves tended to reach saturation as the equilibrium concentrations increased (Figure 4.18). The theoretical Langmuir equation (Eq. 4.2) was also applied with high correlations (Table 4.7) in the case of 2-chlorophenol adsorption. Higher Q° values obtained for the two thermally activated carbons SA4 and PKDA indicated higher adsorption capacities for these carbons compared with chemically activated carbons, as also evidenced from the Freundlich equation. The R_L (dimensionless separation factor) values for each activated carbon (Table 4.7) varied between 0 and 1 indicating a favorable adsorption of 2-chlorophenol on each carbon type. The adsorption of 2-chlorophenol on two thermally activated carbons was more favorable and closer to the irreversible adsorption edge of R_L=0 as evidenced from relatively lower R_L values (Al-Degs et al., 2000). This finding is in agreement with the results of subsequent desorption studies. The R_L values for 2-chlorophenol (Table 4.7) were lower than the ones for phenol (Table 4.2). This finding also indicates that 2-chlorophenol is better adsorbed than phenol and adsorption of 2-chlorophenol is more irreversible than phenol.

Table 4.7. Langmuir isotherm constants for 2-chlorophenol adsorption.

Carbon type	**Q° (mg/g)**	**b (L/mg)**	**R^2**	**R_L**
Norit SA4	250.0	0.0746	1.00	0.191
Norit CA1	140.8	0.0290	0.98	0.556
Norit PKDA	270.3	0.0260	0.95	0.409
Norit CAgran	256.4	0.0069	0.98	0.733

4.2.2. Desorption Studies with 2-Chlorophenol

Desorption equilibrium was determined as 24 hours for each carbon type as seen in Figure 4.20. The Freundlich type isotherm equation (Eq. 4.1) was fitted to desorption data (Figure 4.21) for each carbon type with satisfactory correlations. Desorption Freundlich isotherm constants are shown in Table 4.8. High K values in the case of thermally activated carbons showed that 2-CP loading was still high after desorption. The desorbability of 2-chlorophenol from thermally activated carbons was lower. Desorption capacities were higher for chemically activated carbons CA1 and CAgran as in the previous phenol desorption studies (Section 4.1.2). Also lower 1/n values in the case of thermally activated carbons (SA4 and PKDA) indicated that desorption of 2-CP was more difficult from these carbons and adsorption was highly irreversible. Higher 1/n values obtained for the chemically activated carbons (CA1 and CAgran) indicated that adsorption of 2-CP was more reversible for these carbons. These findings are also supported by Table 4.9, which shows the total adsorption and desorption efficiencies of 2-chlorophenol for each type of activated carbon.

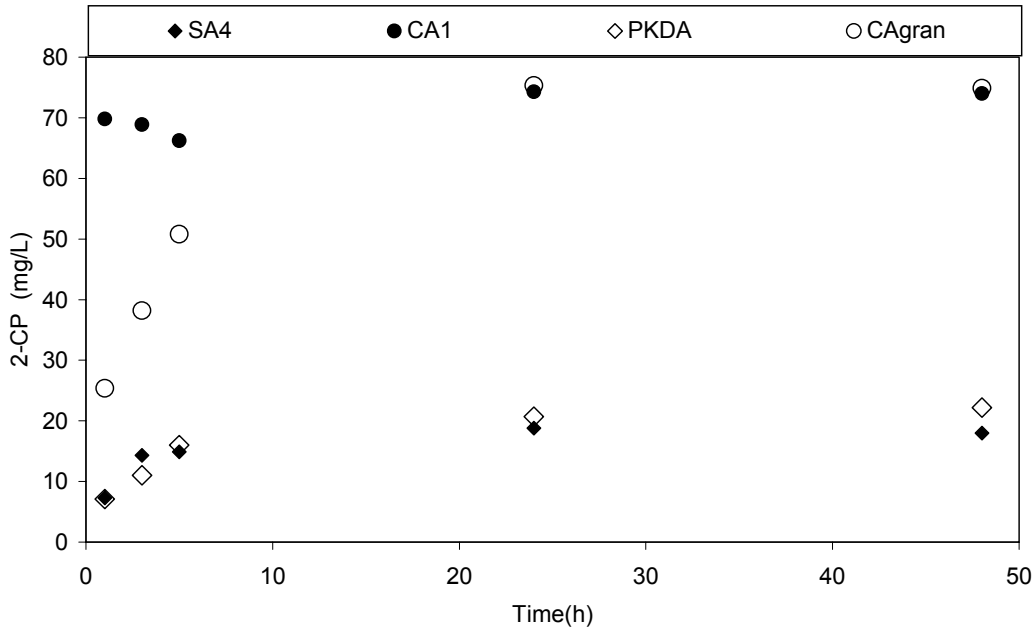


Figure 4.20. 2-Chlorophenol profiles during desorption at a dose of 2 g/L of activated carbons SA4, CA1, PKDA and CAgran.

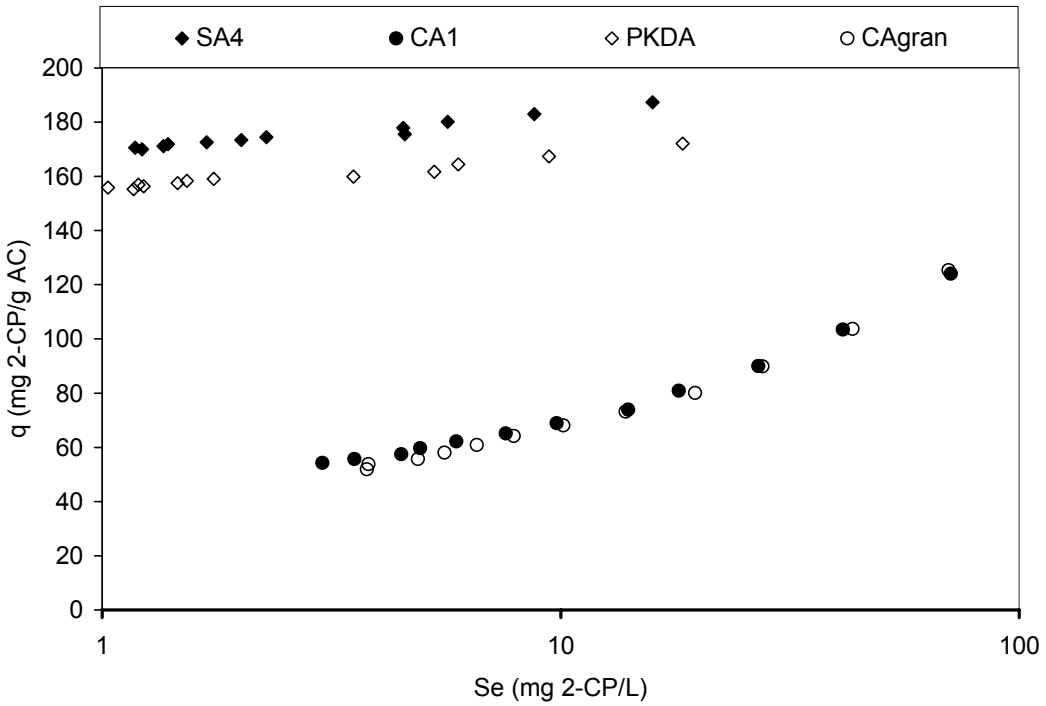


Figure 4.21. 2-Chlorophenol desorption isotherms for activated carbon types SA4, CA1, PKDA and CAgran.

Table 4.8. Freundlich isotherm constants for 2-chlorophenol desorption.

Carbon Type	**Activation method**	**Initial Loading (mg/g)**	**K [(mg/g) (L/mg)$^{1/n}$]**	**1/n**	**R^2**
SA4, powdered	Thermal	195.25	169.2	0.0348	0.97
CA1, powdered	Chemical	159.55	39.3	0.2575	0.99
PKDA, granular	Thermal	181.30	155.4	0.0320	0.95
CAgran, granular	Chemical	160.45	35.4	0.2861	0.99

The isotherm results and the adsorption-desorption efficiencies shown in Table 4.9 reveal that adsorption of 2-chlorophenol is highly irreversible for thermally activated carbons, and highly reversible for chemically activated ones, as in the case of phenol. For all carbon types, 2-chlorophenol was adsorbed with a higher efficiency (Table 4.9) compared with phenol (Table 4.4). This difference was higher in the case of chemically activated carbons. The higher adsorption capacity for 2-CP was attributed to the less polar and soluble nature of chlorinated phenols due to the substitution by a chloride group. Also

larger molecules of chlorinated phenols tend to be more strongly adsorbed than phenol (Vinitnantharat et al., 2001).

On the other hand, reversibility of adsorption was less in the case of 2-chlorophenol (Table 4.9) compared with phenol (Table 4.4). In literature, it was stated that ortho-substituted phenols were more irreversibly adsorbed compared with phenol or para- and meta- substituted phenols (Yonge et al., 1985). Even in the case of chemically activated carbons, reversibility of adsorption was below 68% in the present study (Table 4.9). This is much less than the reversibility of phenol adsorption which was as high as 87% (Table 4.4). Also, for the thermally activated carbons, the adsorption reversibility for 2-chlorophenol (about 13-14 %) was significantly lower than phenol (20-25 %). Higher degrees of hysteresis were calculated for the thermally activated carbons (Table 4.9). This indicates that chemically activated carbons could be preferred for bioregeneration of activated carbons loaded with 2-CP although their adsorption efficiencies were lower.

Table 4.9. Adsorption and desorption efficiencies of 2-chlorophenol for each activated carbon type.

Carbon type	**Carbon Conc. (mg/L)**	**Adsorption Efficiency (%)**	**Desorption Efficiency (%)**	**Irreversible adsorption (%)**	**Degree of hysteresis w (%)**
Norit SA4	2000	80.27	12.94	87.06	459.48
Norit CA1	2000	65.59	66.00	34.00	57.83
Norit PKDA	2000	74.53	14.36	85.64	857.81
Norit CAgran	2000	65.96	67.67	32.33	93.15

The desorption equilibrium curves (Figure 4.20) showed that during the first 5 hours of desorption, more 2-CP was desorbed from PACs compared with their GAC countertypes. Hence, desorption was faster from PACs, just as adsorption was faster onto PACs. The differences between PAC and GAC can be attributed to the particle size. Since GAC particles are much larger than PAC particles, diffusive transport into GAC is slower (Speitel and DiGiano, 1987). Figure 4.22 shows the cumulatively desorbed 2-CP at the end of each equilibrium desorption step and reflects that comparable desorption is achieved from PAC-GAC countertypes. However, slightly higher desorption (Figure 4.22) was obtained for the GACs compared with their powdered countertypes due to their more

macroporous structure (Table 3.1). The differences in cumulative desorption between the powdered and granular countertypes were statistically significant ($p<0.01$ at 95% confidence level). Since 2-chlorophenol was mainly adsorbed on micro- and mesopores, the difference in macropore volume of PAC and GAC countertypes resulted in only a small difference in desorption as in the case of phenol (Section 4.1.2).

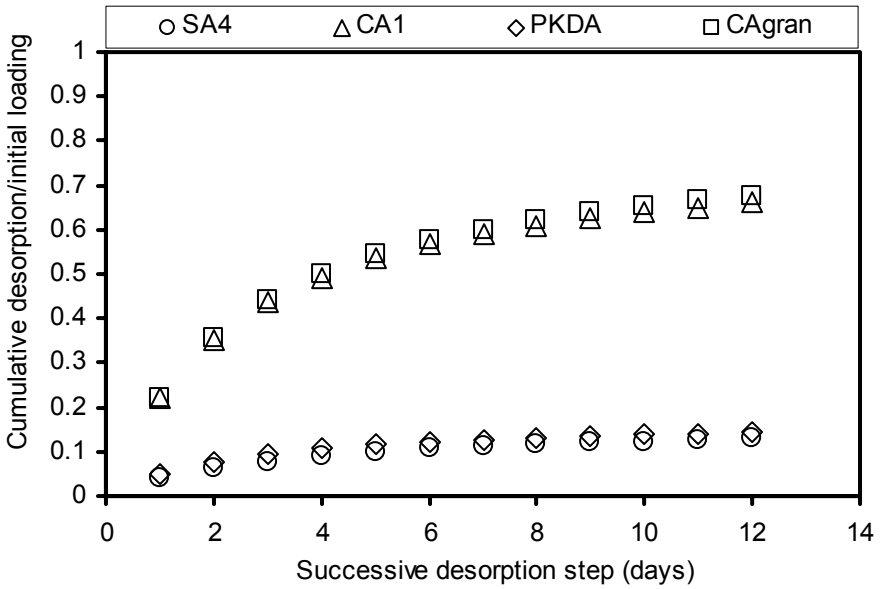


Figure 4.22. Cumulative desorption of 2-chlorophenol from loaded activated carbons at the end of each successive desorption step (SA4: powdered, thermally activated, CA1: powdered, chemically activated, PKDA: granular, thermally activated and CAgran: granular, chemically activated).

4.2.3. Biodegradation and Bioregeneration Studies with 2-Chlorophenol

4.2.3.1. The use of non-acclimated biomass. Initially, non-acclimated biomass was used for biodegradation and bioregeneration in RUN 8 (Table 3.4). After 24 hours aeration, no 2-chlorophenol removal was observed in the biodegradation reactor of RUN 8 (Figure 4.23a). After 72 hours of aeration, the decrease in the initial 2-chlorophenol concentration (384 mg/L) was only 13%, and the decrease in the initial COD concentration (621 mg/L) was only 8%. Between 3rd and 10th days of aeration, 2-chlorophenol and COD concentrations in the biodegradation reactor did not change much. But, at the end of 21 days, the decrease in the initial 2-chlorophenol concentration was 16%, and the decrease in COD was 14% (Figure 4.23a). At such high initial 2-chlorophenol concentrations (384

mg/L), biodegradation of 2-chlorophenol by non-acclimated biomass was not possible in the absence of a growth substrate. Removal was very small and slow (Figure 4.23a). This very small metabolic activity was also concluded from the low oxygen uptake rates shown in Figure 4.23b, which were always below 5 mg/L.h.

2-Chlorophenol and COD profiles in RUN 8 were almost parallel to each other (Figure 4.23a), because the measured COD/2-CP ratio was almost constant (1.66±0.06). This value is very close to the theoretical COD equivalent (1.62) of 2-chlorophenol. This suggested that a small amount of 2-chlorophenol was almost completely mineralized or removed by sorption to activated sludge. Also, further gas chromatographic measurements showed that no phenolic by-products were formed during biodegradation of 2-chlorophenol (Section 4.3.4.1). During mineralization or oxidation of 2-chlorophenol, dechlorination is an expected mechanism, which would lead to an increase of chloride in the bulk solution. Therefore, chloride measurements were performed to determine dechlorination. After 21 days of aeration, a 37 mg/L increase in the chloride ion concentration was measured due to dechlorination of 2-chlorophenol. This revealed that 2-chlorophenol was partly removed by biological activity, although very slowly.

In the bioregeneration reactor in RUN 8, 52 mg/L 2-chlorophenol was desorbed from the loaded activated carbons during the first 24 hours of aeration. This value decreased to 14 mg/L at the end of 21 days aeration (Figure 4.23a). This also showed that the desorbed 2-chlorophenol was biodegraded very slowly. Chloride accumulation of 19 mg/L in the bioregeneration reactor also showed that only a small amount of 2-chlorophenol was dechlorinated.

Efficiency of bioregeneration was determined using the Freundlich adsorption isotherm constants for 2-chlorophenol as explained in Section 4.1.3 for phenol. It was found that 12.3% of the adsorbed 2-chlorophenol was desorbed in the bioregeneration reactor at the end of 24 hours aeration. This ratio is very close to the desorbability efficiency of SA4 for 2-chlorophenol in the absence of biological activity (Table 4.9). During the remaining 20 days of aeration, the desorbed 2-chlorophenol was biodegraded very slowly (Figure 4.23a). Very low OUR values (Figure 4.23b) and low chloride formation (19 mg/L) showed that no further desorption took place during this period. Thus,

under the operating conditions of RUN 8, bioregeneration occurred only due to the initial concentration gradient between the loaded activated carbon and the bulk liquid. Bioregeneration due to exoenzymatic reactions did not seem to be plausible when 2-chlorophenol was the only target compound, although phenol-loaded thermally activated carbons had indicated exoenzymatic bioregeneration (Section 4.1.3.5). The low bioregeneration in the case of 2-chlorophenol can be attributed to both the low desorbability from the carbon SA4 and the slow biodegradation of 2-chlorophenol by the non-acclimated biomass.

4.2.3.2. The use of acclimated biomass. RUN 9 (Table 3.4), in which an acclimated biomass was used and 2-chlorophenol was the only carbon source, also resulted in no significant biodegradation (Figure 4.24). The low biodegradability was also ascertained by OUR values, which were below 5 mg/L.h. However, 2-chlorophenol had been completely removed in the presence of phenol during the acclimation phase (See Section 3.4.3). This showed that 2-chlorophenol was only biodegraded as a co-substrate in the presence of the growth substrate phenol. It can be deduced that this compound can only be biodegraded by a cometabolic mechanism in the activated sludge used in this study. Kim and Hao (1999) extensively investigated the cometabolic degradation of monochlorophenols in the presence of phenol. In their study, biodegradability of chlorophenols decreased as the initial phenol/chlorophenol ratio decreased, indicating that chlorophenols were only removed by a cometabolic pathway in the presence of a sufficient amount of phenol.

In the bioregeneration reactor in RUN 9, activated carbon was not fully loaded with 2-chlorophenol. The purpose was to decrease the amount of 2-chlorophenol in the reactors in order to decrease its inhibitory effects. The 2-CP loading on SA4 carbon was about one third of the loading in RUN 8 (Table 3.4). This resulted in much lower desorption in the bioregeneration reactor (Figure 4.24). In addition to this, biodegradation was very slow. Therefore, in the case of 2-chlorophenol, bioregeneration did not occur even with the acclimated biomass. In previous phenol studies (Section 4.1.3), phenol was highly biodegradable, even when the same non-acclimated biomass was used as in this study, and all four types of activated carbon were successfully bioregenerated to some extent (Table 4.5). The results for 2-chlorophenol and phenol showed that the type of the target compound was very important for the extent of bioregeneration.

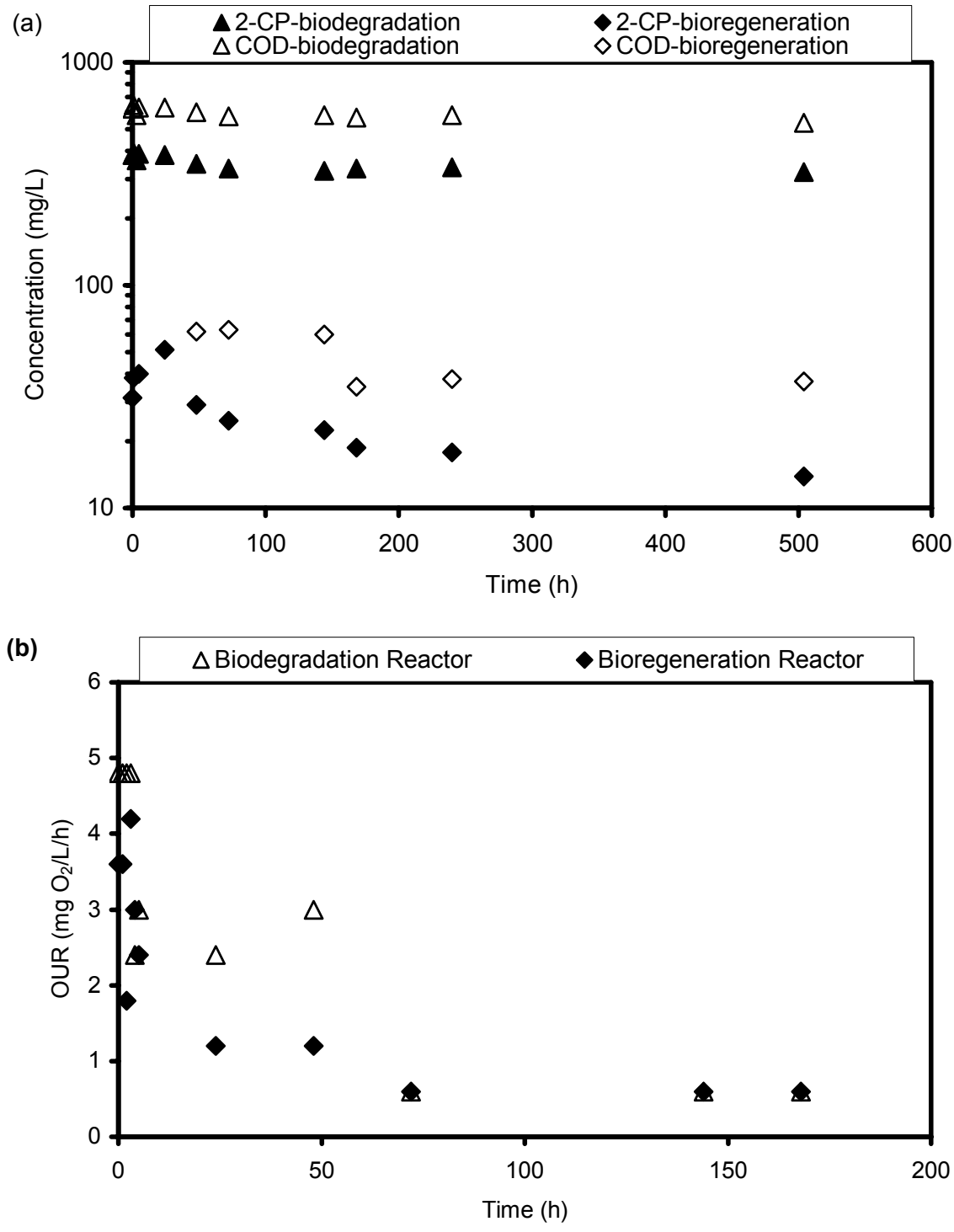


Figure 4.23. a) 2-chlorophenol and COD profiles and b) OUR profiles in biodegradation and bioregeneration reactors in RUN 8 with 2-chlorophenol when non-acclimated biomass and SA4 carbon were used.

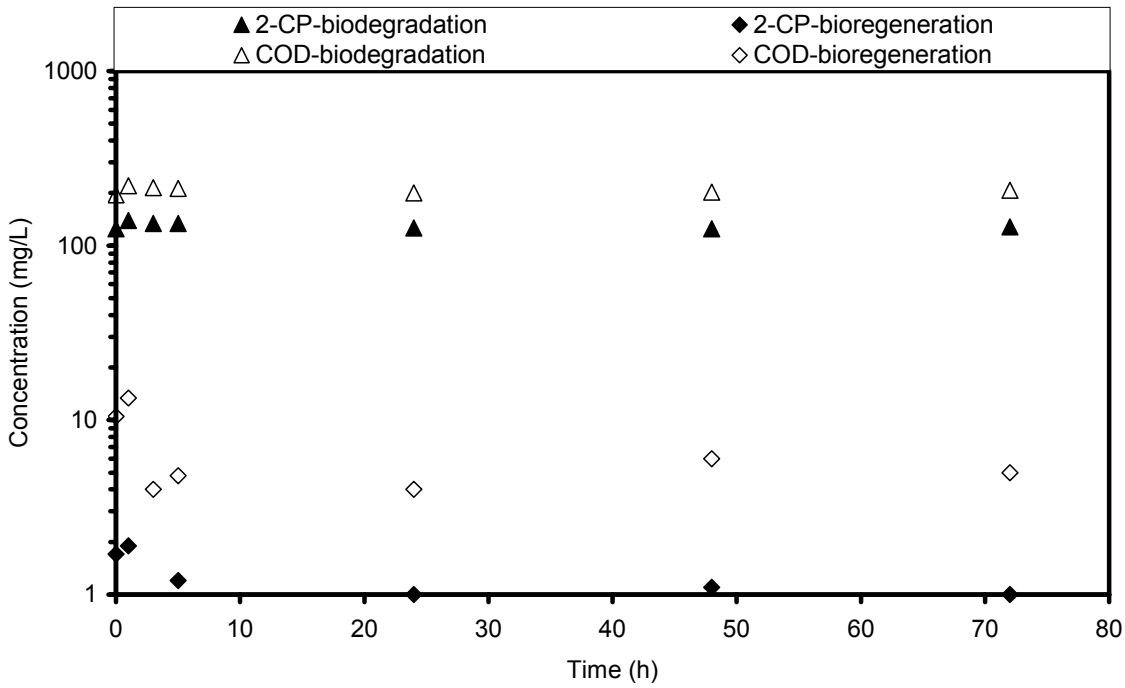


Figure 4.24. 2-chlorophenol and COD profiles in biodegradation and bioregeneration reactors in RUN 9 with 2-chlorophenol when acclimated biomass and SA4 carbon were used.

4.3. Experiments with a Bi-solute Mixture of Phenol and 2-Chlorophenol

4.3.1. Competitive Adsorption Studies

The isotherm data for the competitive adsorption of phenol and 2-chlorophenol on each activated carbon type are plotted in Figure 4.25 as closed data points. In order to compare competitive adsorption with single-solute adsorption, the previously obtained single-solute adsorption data (Sections 4.1.1 and 4.2.1) were also included in Figure 4.25 as open data points. The competitive adsorption data for phenol did not fit the Freundlich equation for any of the activated carbon types although previous single-solute data had satisfied this condition (Section 4.1.1). The competitive adsorption data for 2-CP fitted the Freundlich equation (Eq. 4.1), as in the previous single-solute adsorption isotherms with this compound (Section 4.2.1). The Freundlich adsorption isotherm constants for 2-chlorophenol are shown in Table 4.10.

The K value, which is an indicator of adsorption capacity, was lower for the competitive adsorption of 2-CP compared with the single-solute adsorption for each type of activated carbon. Previous single-solute 2-CP adsorption studies had resulted in K [(mg/g) $(L/mg)^{1/n}$] values of 86.51 for SA4, 13.58 for CA1, 60.4 for PKDA and 18.4 for CAgran (Table 4.6). The adsorption intensity, 1/n, was shown by slope of the adsorption isotherms (Figure 4.25). In competitive adsorption, 1/n values were higher (Table 4.10) compared with the previous single-solute values of 0.1947 for SA4, 0.4064 for CA1, 0.2265 for PKDA and 0.5526 for CAgran. This indicated that an increase in the activated carbon dose was slightly more effective in the case of single-solute adsorption than in bi-solute adsorption. In both single-solute and bi-solute adsorption, the thermally activated carbons were obviously better adsorbers for 2-chlorophenol, as shown by higher K and lower n values (Table 4.6). This was mainly attributed to the changes in surface chemical properties, which were related to the type of activation in Section 4.1.1. Chemical treatment of carbons CA1 and CAgran with phosphoric acid resulted in an acidic surface. Acidic surface properties are associated with the formation of oxygen-containing surface functional groups and higher polarity. This leads to water adsorption by means of hydrogen bonding on oxygen-containing functional groups, and clustering of additional water molecules around adsorbed water molecules. Such water clusters can prevent pollutant

access to hydrophobic regions on the carbon surface, reduce interaction energy between the pollutant and carbon surface and block pollutant access to micropores (Knappe et al., 2003; Puri, 1980). The negative influence of surface oxides has been also attributed to the depletion of the electronic π-band of graphite-like layers resulting in consequent lowering of van der Waals forces of interaction (Puri, 1980) and also reduced oxidative polymerization of phenols (Garcia-Araya et al., 2003).

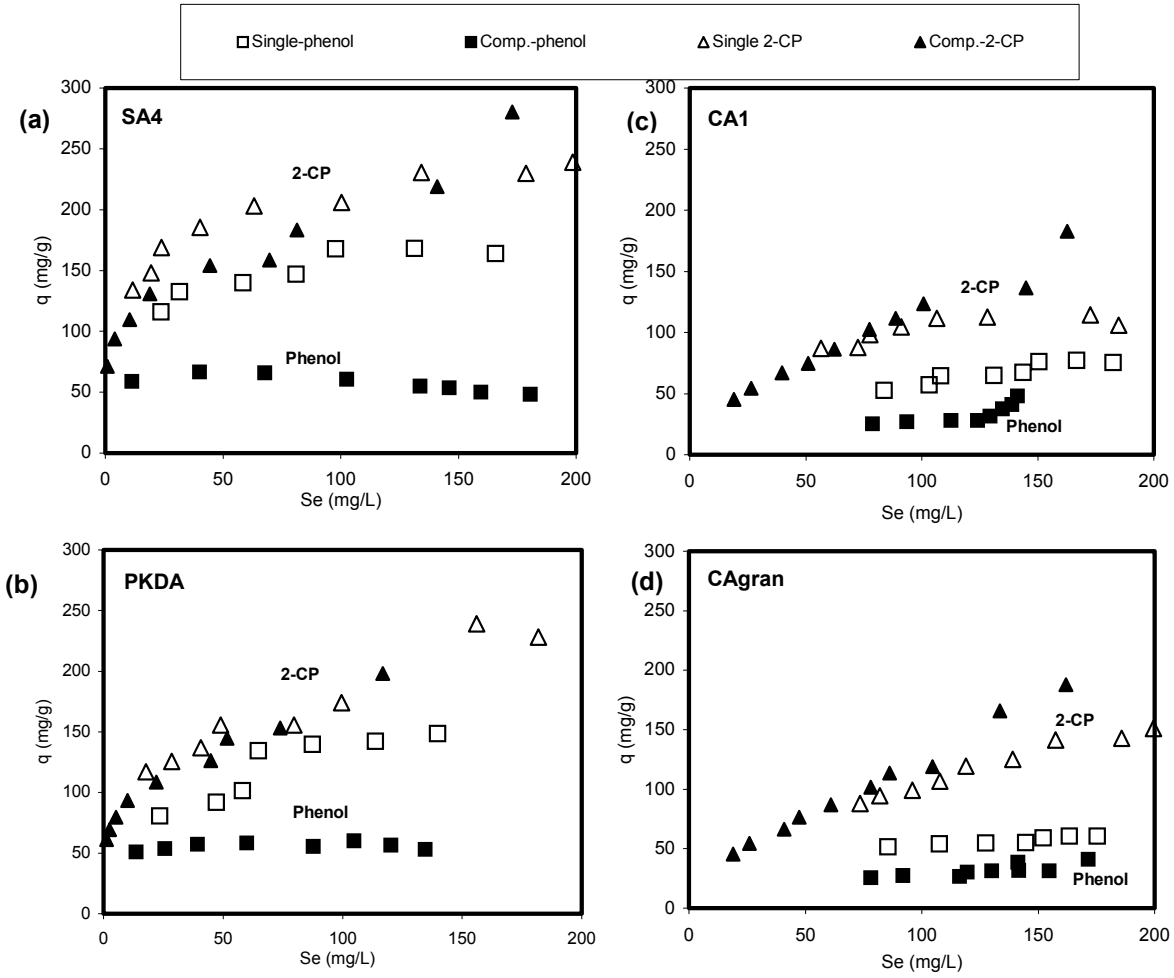


Figure 4.25. Competitive bi-solute and single-solute adsorption isotherms for phenol and 2-CP for a) SA4, b) PKDA, c) CA1, d) CAgran. (open data points refer to single-solute adsorption, closed data points refer to bi-solute adsorption).

Figure 4.26 illustrates the effect of carbon dose on equilibrium bulk phenol and 2-CP concentrations, both in the cases of single-solute and competitive adsorption. For each carbon type, 2-CP adsorption was not negatively affected by the presence of phenol.

However, phenol adsorption was suppressed to a great extent in the competitive presence of 2-CP (Figures 4.25 and 4.26). Similar adsorption of 2-CP in bi-solute and single-solute systems showed that 2-CP was preferentially adsorbed, but phenol was adsorbed competitively. Also according to Sontheimer et al. (1985), the better adsorbable compound influences the adsorbability of the weakly adsorbable one more than the opposite. In such a case, adsorption data of the weakly adsorbable compound takes a curved shape (Sontheimer et al., 1985), as seen in the case of phenol in this study (Figure 4.25). This points out the presence of a preferentially adsorbed compound, which is 2-CP in this case. The shape of 2-CP data was not curved, which showed preferential adsorption. According to Sontheimer et al. (1985), the higher 1/n values for 2-CP in the bi-solute case also showed that there was still competition between phenol and 2-CP although 2-CP was adsorbed preferentially.

Table 4.10. Competitive adsorption Freundlich isotherm constants for 2-chlorophenol in the presence of phenol.

Carbon type	**Physical form**	**Activation method**	**K [(mg/g) (L/mg)$^{1/n}$]**	**1/n**	**R^2**
Norit SA4	Powdered	Thermal	66.3	0.2387	0.95
Norit CA1	Powdered	Chemical	7.2	0.6113	0.98
Norit PKDA	Granular	Thermal	54.6	0.2435	0.97
Norit CAgran	Granular	Chemical	6.3	0.6528	0.98

According to the Polanyi-Manes adsorption potential theory, the conditions for adsorption were satisfied only for one adsorbate (Knettig et al., 1986), which was 2-CP in this study. Moreover, at low carbon dosages resulting in higher 2-CP equilibrium concentrations, the bi-solute adsorption of 2-CP exceeded single-solute adsorption, best seen for CA1 and CAgran (Figure 4.25). This can be attributed to the interaction effects between phenolic adsorbates, which are reported to favour the adsorption of one component (Garcia-Araya et al., 2003). Similar results were reported in a previous study (Fritz et al., 1980) for the *p*-nitrophenol and phenol couple, where *p*-nitrophenol was preferentially adsorbed. Also in that study, there was only a weak reduction in *p*-nitrophenol adsorption capacity, whereas phenol adsorption was very much reduced due to competition (Fritz et al., 1980). However, Knettig et al. (1986) had previously reported

non-competitive adsorption for both phenol and 2-CP, probably due to the carbon type and concentration range studied.

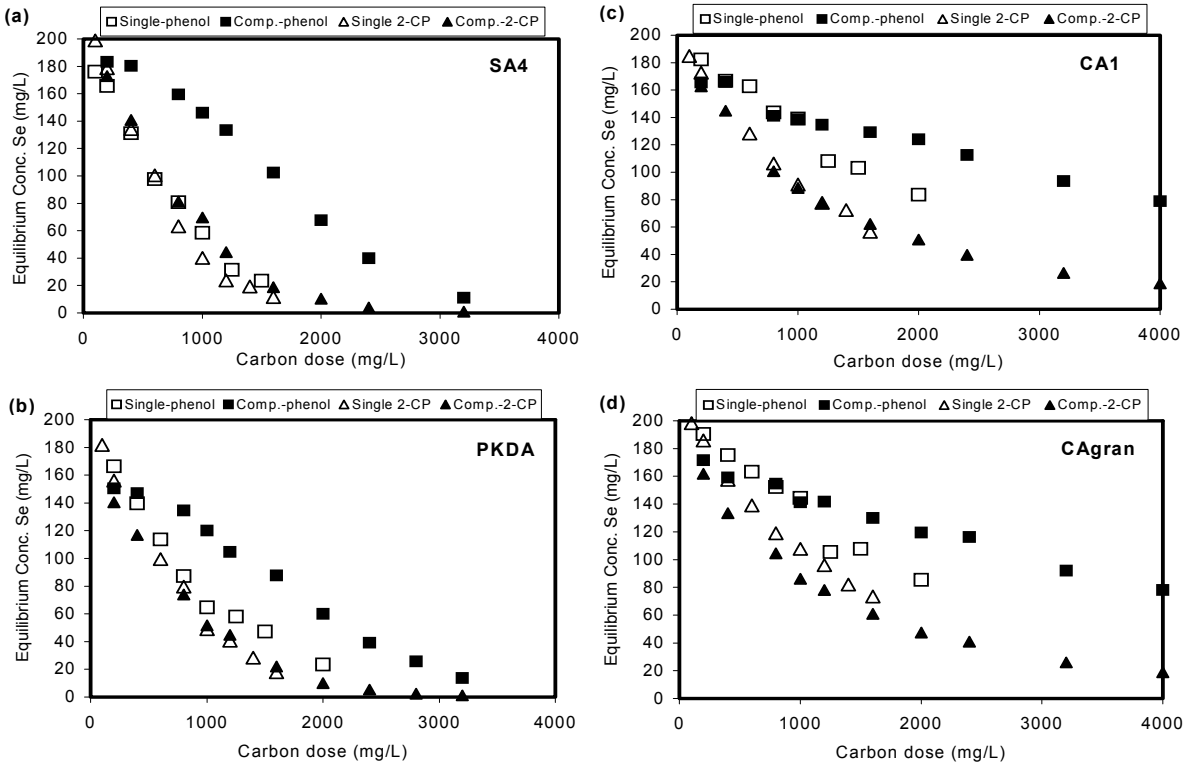


Figure 4.26. Effect of activated carbon dose on equilibrium phenol and 2-CP concentrations in competitive bi-solute and single-solute adsorption studies with initial concentrations of 200 mg/L for a) SA4, b) PKDA, c) CA1, d) CAgran. (open data points refer to single-solute adsorption, closed data points refer to bi-solute adsorption).

The difference between single-solute and bi-solute adsorption of phenol was much more obvious in the case of thermally activated carbons SA4 and PKDA. These carbons adsorbed phenol much better than chemically activated ones when it was present as a single-solute (Section 4.1.1). The preferential adsorption of 2-CP by the thermally activated carbons resulted in a drastic decrease in phenol adsorption. Previously, it was shown that the more hydrophobic 2-CP was adsorbed better than the more hydrophilic phenol (Section 4.2.1). In addition to that finding, bi-solute studies showed that 2-CP was adsorbed preferentially by each type of activated carbon. Knettig et al. (1986) suggested that the enhanced preferential adsorption of chlorinated compounds would be beneficial for mixed phenolic systems since they are more noxious and resistant to biodegradation.

Although 2-CP adsorption did not decrease much in the presence of phenol, cumulative adsorption of phenol and 2-CP increased in the bi-solute adsorption since some phenol was also adsorbed. In addition to 2-CP adsorption, phenol adsorption was 57.4±6.9 mg/g for SA4, 33.3±8.1 mg/g for CA1, 55.7±3.0 mg/g for PKDA and 31.5±5.3 mg/g for CAgran. In the case of competitive adsorption, phenol loadings were still higher for thermally activated carbons than chemically activated ones. This enhancement of cumulative adsorption for bi-solute mixtures of aromatic compounds was also observed in other studies, generally for phenol and substituted phenols. It was mainly attributed to the influence of differences in molecular size (Garner et al., 2001). However, there are also studies reporting that cumulative adsorption capacities for a mixture of phenolic compounds were lower than those for individual compounds (Knettig et al., 1986).

The theoretical Langmuir equation (Eq 4.2) was also tested in adsorption. Competitive 2-CP adsorption fitted the Langmuir equation with satisfactory correlations (Table 4.11), but competitive phenol adsorption fitted the Langmuir equation only for thermally activated carbons (SA4 and PKDA) and did not fit for chemically activated ones (CA1 and CAgran). The higher Q° values for 2-CP adsorption also indicated higher adsorption of 2-CP compared with phenol. Q° values for bi-solute phenol adsorption by SA4 and PKDA in Table 4.11 were much lower than the previously obtained single-solute Q° values of 178.6 mg/g for SA4 and 204.1 mg/g for PKDA (Table 4.2). Q° represents a practical limit of adsorption capacity. Hence, the maximum adsorption capacity for phenol decreased in the case of bi-solute adsorption. Although a good correlation was obtained with Langmuir equation (Table 4.11), the decrease in Q° and negative b values showed that a monolayer coverage of carbon surface did not occur for bi-solute adsorption of phenol.

Table 4.11. Competitive Langmuir adsorption isotherm constants for phenol and 2-CP .

	Carbon	**Q° (mg/g)**	**b (L/mg)**	**R^2**	**R_L**
Phenol	SA4	47.6	-0.0826	0.98	n.a.
	CA1	n.a.	n.a.	n.a.	n.a.
	PKDA	55.6	-1.3740	0.99	n.a.
	CAgran	n.a.	n.a.	n.a.	n.a.
2-CP	SA4	263.2	0.0500	0.92	0.249
	CA1	212.8	0.0122	0.96	0.658
	PKDA	192.3	0.0892	0.95	0.228
	CAgran	200.0	0.0138	0.96	0.643

n.a.: not applicable

The R_L (dimensionless separation factor) values (Eq. 4.4) for 2-CP (Table 4.11) varied between 0 and 1 indicating a favorable adsorption of 2-CP on each carbon type. The adsorption of 2-CP on two thermally activated carbons was more favorable and closer to the irreversible adsorption edge of R_L=0, as evidenced from relatively lower R_L values. These findings are in agreement with the results of subsequent desorption studies in Section 4.3.3.

4.3.2. Description of Competitive Adsorption Using Models

The loadings on activated carbon (q) in the case of competitive adsorption can be modelled by using the single-solute adsorption parameters. The Ideal Adsorbed Solution (IAS) model originally developed by Myers and Prausnitz (1965) to describe competitive adsorption of gases was extended by Radke and Prausnitz (1972) to aqueous mixtures of solutes. DiGiano et al. (1980) provided a Simplified Ideal Adsorbed Solution (SIAS) model to simplify the handling of mathematical equations. According to the SIAS model, the individual loadings in the mixture can be calculated from Equation 4.14.

$$q_i = K'^{\left(\frac{n'-1}{n'}\right)} \left[K_i C_i^{\,n_i}\right]^{1/n'} \left[\sum_N \left(\frac{K_i}{K'} C_i^{\,n_i}\right)^{1/n'}\right]^{(n'-1)} \tag{4.14}$$

where

q_i = solid-phase equilibrium concentration of solute i,

K_i, n_i = Single-solute empirical Freundlich constants for solute i,

C_i = liquid-phase equilibrium concentration of solute i,

n’ = average value of n_i, and

K’ = average value of K_i.

The SIAS model was modified by Yonge and Keinath (1986) and the Improved Simplified Ideal Adsorbed Solution (ISIAS) model was developed in order to account for non-ideal competition for adsorption sites by adding a competition factor, a. This model equation can be seen in Equation 4.15.

$$q_i = K'^{\left(\frac{n'-1}{n'}\right)} \left[\frac{K_i}{a_i} C_i^{\,n_i} \right]^{1/n'} \left[\sum_N \left(\frac{K_i/a_i}{K'} C_i^{\,n_i} \right)^{1/n'} \right]^{(n'-1)} \tag{4.15}$$

where

$$K' = \frac{\sum (K_i/a_i)}{N}$$

The single-solute Freundlich parameters obtained in Sections 4.1.1 and 4.2.1 were used to predict the competitive bi-solute loadings using both the SIAS and ISIAS models. The SIAS model could not predict the experimentally measured loadings for each activated carbon type, although it could roughly estimate the trend of the actual curve (Figure 4.27). The SIAS model underestimated 2-CP loadings on each type of activated carbon. However, the model overestimated phenol loadings, particularly those on thermally activated carbons SA4 and PKDA (Figure 4.27a,b), whereas this overestimation was not obvious for chemically activated carbons CA1 and CAgran (Figure 4.27c,d). These findings showed that competition between phenol and 2-CP was non-ideal, but in the direction to favor 2-CP adsorption. Although the single-solute adsorption data had indicated higher adsorbability of 2-CP compared with phenol, much more adsorption of 2-CP took place in bi-solute mixture than predicted from single-solute adsorption. Besides the ideal adsorbed solution theory, the bi-solute data were also investigated using the Langmuir competitive model as described by Singer and Yen (1980). The Langmuir model also could not predict the actual bi-solute data and resulted in overestimation of phenol and underestimation of 2-CP adsorption (data not shown). This was attributed to the fact that the competitive Langmuir model does not have a thermodynamic basis, and the assumptions of a homogeneous surface and uniform adsorption are not valid for activated carbon (Singer and Yen, 1980).

The non-ideal competition between phenol and 2-CP necessitated the use of the ISIAS model. The ISIAS model requires the determination of optimum a_i values in Eq. 4.15, where a_1 and a_2 refer to the competition factors for phenol and 2-CP, respectively. The optimum a_1 and a_2 values were determined by nonlinear programming which provides the lowest residual sum of squares (RSS) of competitive bi-solute data. RSS is the sum of squares of the error (difference between the experimental and model q values). The

optimum a_1 and a_2 values and corresponding to lowest RSS values (divided by the number of data points) for each type of carbon are presented in Table 4.12.

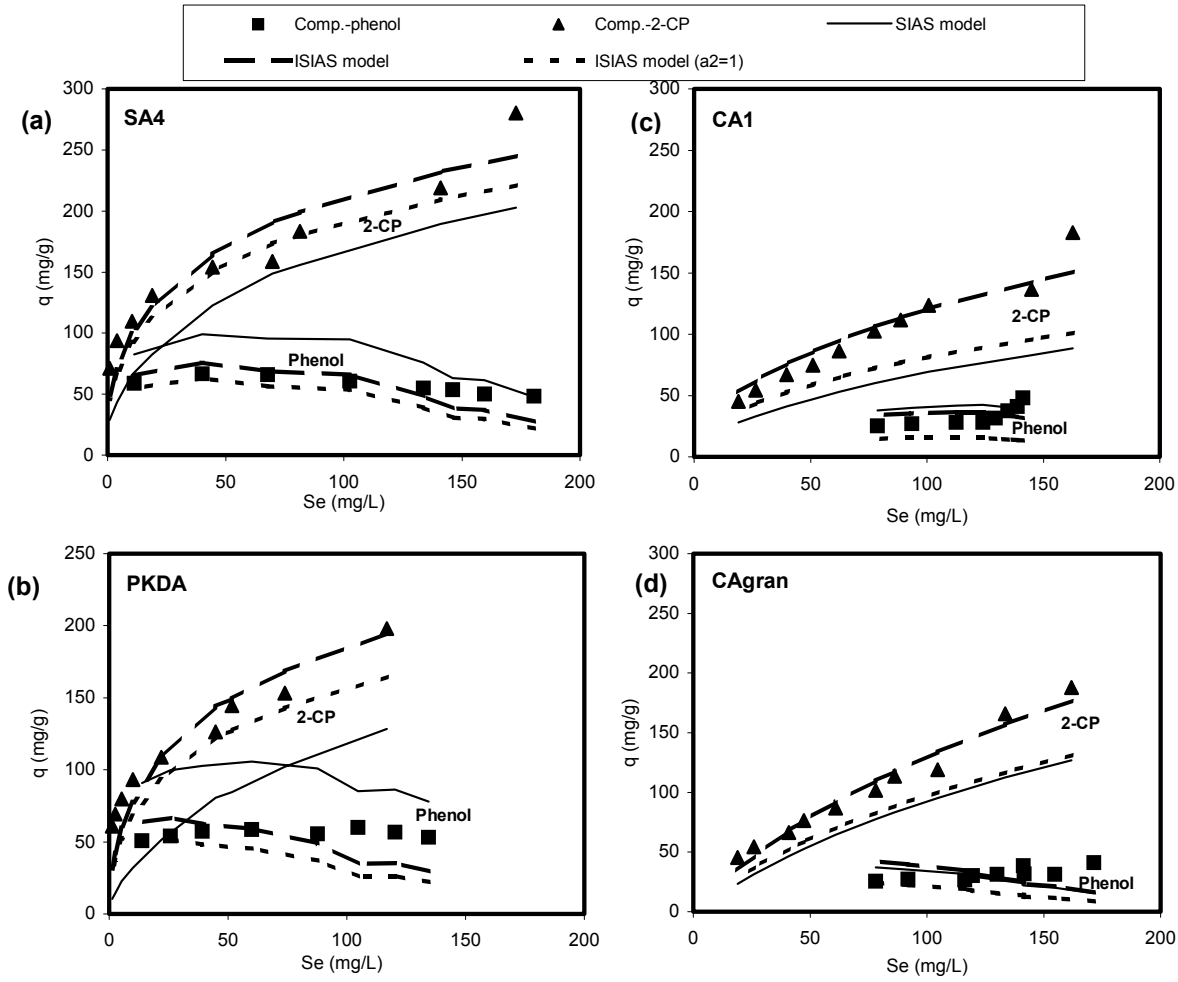


Figure 4.27. Competitive bi-solute isotherms for phenol and 2-CP with their Simplified Ideal Adsorbed Solution (SIAS) and Improved SIAS (ISIAS) model fits for a) SA4, b) PKDA, c) CA1, d) CAgran. (full lines refer to SIAS model fits, dashed lines refer to ISIAS model fits).

The ideal adsorbed solution theory (IAST) assumes that adsorption sites are equally accessible to both solutes and adsorption is reversible (Knappe et al., 2003). The competition factors reported as a_i values in Table 4.12 denote deviations from IAST. The a_i values distant from 1 indicate that the competitive adsorption becomes more distant from ideal competition. Particularly, a_2 values of 2-CP adsorption were much smaller for chemically activated carbons (CA1 and CAgran) compared with thermally activated

carbons (SA4 and PKDA). This showed that competitive adsorption of 2-CP on chemically activated carbons was more distant from ideality in favor of 2-CP adsorption.

Table 4.12. ISIAS model competition factors for phenol and 2-CP .

Carbon type	**a_1 (phenol)**	**a_2 (2-CP)**	**RSS/data points (mg^2/g^2)**	**a_1 (a_2=1)**	**RSS/data points (a_2=1) (mg^2/g^2)**
SA4	1.030	0.894	300	1,179	469
CA1	0.900	0.645	118	n.a.	n.a.
PKDA	1.132	0.836	286	1.392	525
CAgran	0.817	0.732	116	n.a.	n.a.

a_1 and a_2: competition factors in Eq. 4.15 for phenol and 2-CP, respectively.
n.a.: not applicable

Different degrees of irreversible adsorption were previously reported both for phenol and 2-CP as single-solutes (Tables 4.4 and 4.9) and also for bi-solute adsorption (see Section 4.3.3). This irreversible adsorption is a reason for the failure of the IAST as well as the unequal competition for adsorption sites (Yonge and Keinath, 1986). The best fit of the SIAS model was seen in the case of phenol adsorption on chemically activated carbons (Figure 4.27) due to highly reversible adsorption (see Section 4.3.3).

Although the ISIAS model provided a reasonable fit for the bi-solute data, the improvement in the IAST obtained by the ISIAS model was the result of parameters that were determined from the bi-solute data itself. In determination of optimum a_1 and a_2 values in Eq. 4.15, bi-solute adsorption data were also required besides the single-solute adsorption parameters. Yonge and Keinath (1986) suggested a correlation between competition factors and the solubility of competing solutes in order to make the model truly predictive without the use of bi-solute data. They stated that the term associated with the low solubility sorbate (a_2 of 2-CP in this case) was not significant and should be taken as equal to 1, and the term associated with the higher solubility sorbate (a_1 of phenol in this case) resulted in a significant improvement in data description. Based on the inverse relationship between solubility and adsorbability, the authors developed a correlation between a_1 and $(\delta_1-\delta_2)/\ \delta_1$, where δ= sorbate solubility and $\delta_1>\delta_2$.

However, the results of the present study showed that this modification of the ISIAS model was not successful in describing the bi-solute data for chemically activated carbons

used in this study (Figure 4.27c,d). Calculated a_1 values were not reliable and therefore were not tabulated in Table 4.12 for CA1 and CAgran. This showed that this modification of the ISIAS model is not suitable for every type of activated carbon. The modified ISIAS model gave a better fit for the thermally activated carbons SA4 and PKDA (Figure 4.27a,b), but a_1 values with the assumption of a_2=1 were not actually the same for SA4 and its granular countertype PKDA (Table 4.12). The numerical value of $(\delta_1-\delta_2)/\ \delta_1$ was approximately equal to 0.67 for the phenol (δ_1=8.45 %) and 2-CP couple (δ_2=2.8 %). The empirical relationship between a_1 and $(\delta_1-\delta_2)/\ \delta_1$, as shown by Yonge and Keinath (1986), was a very rough estimate and was generalized for aromatic compounds. Data presented by Yonge and Keinath (1986) can be used to develope an empirical equation (Eq. 4.16) for a very rough calculation of a_1.

$$a_1 \cong 0.69 * (((\delta_1-\delta_2)/\ \delta_1)-0.26) + 1.035 \qquad (4.16)$$

Using the empirical equation above, a_1 was calculated to be about 0.132 for the phenol and 2-CP couple. This value was not equal to the a_1 values obtained in our study, but also was not very distinct from them. Thus, such an empirical relationship between the competition factor and solubilities of solutes may not result in perfect estimations of the competitive data, but can only give a rough idea about it. As also evidenced from Figure 4.27 and lower RSS values reported in Table 4.12, a_1 and a_2 values calculated according to the ISIAS model could give better predictions compared with the modified ISIAS model for the concentration ranges used in this study. However, the differences in a values in Table 4.12 showed that these should be calculated for each type of carbon. This is necessary because of different activation methods, surface chemical properties, porosities etc. of every carbon type.

4.3.3. Desorption Studies

Bi-solute desorption data for each carbon type were fitted into the Freundlich type isotherm equation (Eq. 4.1) with satisfactory correlations. However, desorption data did not fit the Langmuir equation (Eq. 4.2). Desorption isotherms are shown in Figure 4.28 and desorption Freundlich isotherm constants are shown in Table 4.13. High K values for thermally activated carbons SA4 and PKDA showed that both phenol and 2-CP loadings

were still high after desorption. In the bi-solute case, desorbability of these compounds from thermally activated carbons was lower, as in the case of single-solute desorption (Sections 4.1.2 and 4.2.2). Also, lower 1/n values indicated that desorption was more difficult from these carbons. Desorption capacities were higher for the chemically activated carbons CA1 and CAgran both for phenol and 2-CP (Figure 4.29).

Table 4.13. Freundlich desorption isotherm constants for competitively adsorbed phenol and 2-CP.

	Carbon type	**Initial Loading (mg/g)**	**K [(mg/g) (L/mg)$^{1/n}$]**	**1/n**	**R^2**
phenol	SA4	67.13	45.4	0.0505	0.94
	CA1	38.31	0.3	1.0571	0.87
	PKDA	62.56	41.5	0.0501	0.91
	CAgran	39.88	0.3	1.1390	0.88
2-CP	SA4	93.81	89.7	0.0110	0.92
	CA1	69.50	31.0	0.1459	0.91
	PKDA	86.73	80.6	0.0125	0.87
	CAgran	69.45	25.8	0.2127	0.97

In single-solute systems, phenol loadings were as 149.35 mg/g for SA4, 74.6 mg/g for CA1, 120.35 mg/g for PKDA, 75.8 mg/g for CAgran (Table 4.3) and 2-CP loadings were as 195.25 mg/g for SA4, 159.55 mg/g for CA1, 181.3 mg/g for PKDA, 160.45 for CAgran (Table 4.8). Competitive adsorption of phenol and 2-CP in the initial phase of loading of carbons with these compounds resulted in lower phenol and 2-CP loadings (Table 4.13) compared with the single-solute loadings. The reduction in the initial loading resulted in lower K values in the case of bi-solute desorption (Table 4.13). The previously obtained single-solute K [(mg/g) (L/mg)$^{1/n}$] values were as 120.46 for SA4, 8.31 for CA1, 89.29 for PKDA, 9.07 for CAgran in the case of phenol (Table 4.3) and as 169.21 for SA4, 39.29 for CA1, 155.36 for PKDA, 35.42 for CAgran in the case of 2-CP (Table 4.8). The decrease in K in the bi-solute desorption of both compounds was more notable in the case of thermally activated carbons, which are prone to higher irreversible adsorption. This indicated that competition between phenol and 2-CP was severe for adsorption to more energetically active chemisorption sites. Previously, chemisorption was suggested as the dominant adsorption mechanism for the thermally activated carbons SA4 and PKDA (Section 4.1.2).

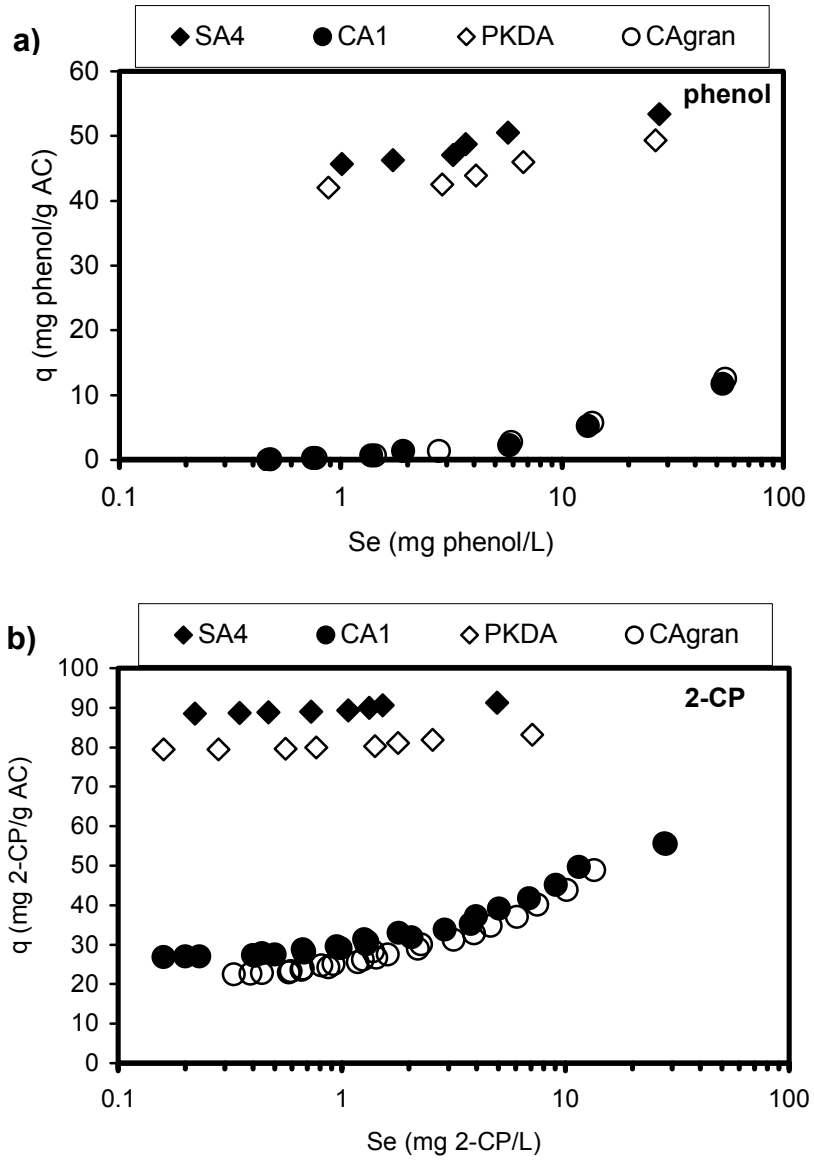


Figure 4.28. Competitive desorption isotherms of each activated carbon type for a) phenol and b) 2-CP.

On the other hand, 1/n values in bi-solute phenol desorption (Table 4.13) were higher than single-solute 1/n values for phenol, which were reported as 0.0242 for SA4, 0.307 for CA1, 0.0358 for PKDA, 0.2158 for CAgran (Table 4.3). However, 1/n values in bi-solute 2-CP desorption (Table 4.13) were lower than single-solute 1/n values for 2-CP reported previously as 0.0348 for SA4, 0.2575 for CA1, 0.032 for PKDA, 0.2861 for CAgran (Table 4.8). This showed that phenol was desorbed more easily in the bi-solute case, but desorption of 2-CP was more difficult in this case for each type of activated carbon.

Therefore, it is suggested that 2-CP was preferentially adsorbed on highly energetic adsorption sites. On the other hand, phenol was adsorbed on less energetic sites with smaller adsorption energies, so that it was more readily desorbed from each carbon type.

This finding was also supported by much higher desorption efficiencies for phenol, although adsorption efficiencies were lower for phenol compared with 2-CP (Table 4.14). Phenol adsorption efficiencies in Table 4.14 were also much lower than previous single-solute adsorption efficiencies of 63.1 % for SA4, 31.5% for CA1, 50.9 % for PKDA, 32% for CAgran (Table 4.4). However, bi-solute 2-CP adsorption efficiencies (Table 4.14) were close to the single solute adsorption efficiencies of 80.3 % for SA4, 65.6 % for CA1, 74.5 % for PKDA, 66 % for CAgran (Table 4.9). These findings are in agreement with adsorption studies, which showed that 2-CP adsorption was not negatively affected in the presence of phenol. On the other hand, phenol desorption efficiencies in Table 4.14 were much higher than the single-solute desorption efficiencies of 20.3 % for SA4, 86.6 % for CA1, 25.8 % for PKDA, 87.5 % for CAgran (Table 4.4). Contrary to that, bi-solute 2-CP desorption efficiencies (Table 4.14) were lower than the single solute desorption efficiencies of 12.9 % for SA4, 66 % for CA1, 14.4 % for PKDA, 67.7 % for CAgran (Table 4.9).

Table 4.14. Competitive adsorption and desorption efficiencies for bi-solute mixtures of phenol and 2-CP.

	Carbon type	**Adsorption Efficiency (%)**	**Desorption Efficiency (%)**	**Irreversible adsorption (%)**	**Degree of hysteresis w (%)**
phenol	SA4	33.43	31.87	68.13	n.a.
	CA1	19.08	99.9	0.1	n.a.
	PKDA	31.16	32.75	67.25	n.a.
	CAgran	19.86	99.92	0.08	n.a.
2-CP	SA4	86.23	5.68	94.32	2070
	CA1	63.88	61.33	38.67	319
	PKDA	79.72	8.43	91.57	1848
	CAgran	63.84	67.72	32.28	207

n.a. : not applicable because phenol adsorption did not fit the Freundlich equation.

The degree of hysteresis is an indicator for adsorption reversibility, which bases on the difference between adsorption and desorption intensities. The lower reversibility of 2-CP adsorption in the bi-solute case was also shown by the calculation of the degree of hysteresis (w) using Equation 4.5. Higher degrees of hysteresis were calculated for thermally activated carbons (Table 4.14). These were also higher in bi-solute desorption of 2-CP (Table 4.14) compared with the single-solute values of 459 % for SA4, 58 % for CA1, 858 % for PKDA, 93 % for CAgran (Table 4.9). The degree of hysteresis could not be calculated for phenol, because phenol adsorption did not fit the Freundlich equation.

Figure 4.29 shows the cumulative amount of desorbed phenol and 2-CP at the end of each equilibrium desorption step and reflects that comparable desorption is achieved from PAC-GAC countertypes. However, slightly higher desorption (Figure 4.29) was obtained for the GACs compared with their powdered countertypes due to their more macroporous structure (Table 3.1). This difference was significant at a level of 95% confidence except for phenol desorption from chemically activated carbons. When this finding is combined with the comparable adsorbabilities on PAC and GAC countertypes, it can be deduced that both phenol and 2-CP were mainly adsorbed on micro- and mesopores rather than macropores. But, the physical adsorption on macropores was not negligible and still resulted in statistically significant differences, except in the case of phenol adsorption on chemically activated carbons.

Figure 4.29 showed that complete desorption of phenol from all carbon types and that of 2-CP from the thermally activated carbons occurred within 6-8 desorption steps. However, complete desorption of 2-CP from the chemically activated carbons occurred within 25 desorption steps, although the cumulative desorption was higher from these carbons. In the case of thermally activated carbons, 2-CP was mainly adsorbed chemically. Those chemically adsorbed 2-CP molecules did not desorb. But physically adsorbed 2-CP, which contributes to a very small part in total, was readily desorbed (Figure 4.29). On the other hand, most of the adsorbed 2-CP was physically adsorbed on chemically activated carbons. The van der Waals forces, which resulted in physical adsorption, could be broken down. But, it probably required more desorption steps, particularly for 2-CP adsorbed on narrower micropores. Desorption of phenol occurred faster, obviously because phenol was adsorbed with a weaker energy than 2-CP as discussed earlier.

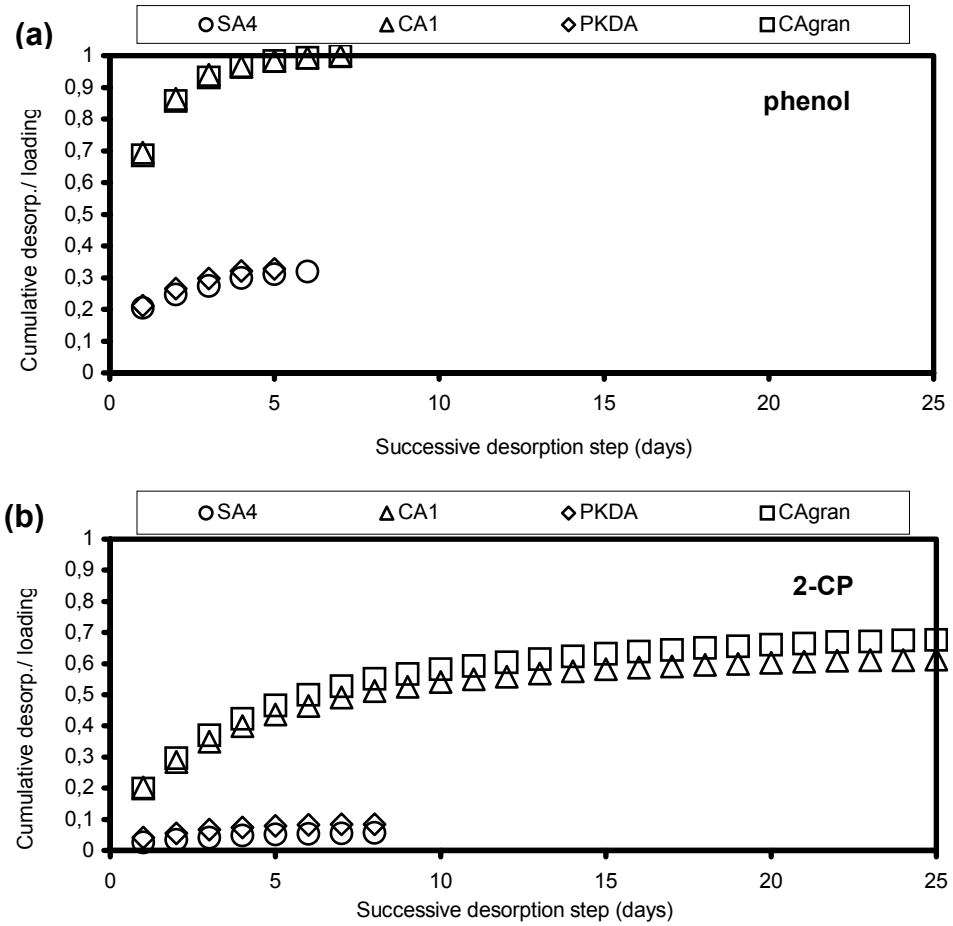


Figure 4.29. Cumulative desorption of a) phenol and b) 2-CP from competitively loaded activated carbons at the end of each successive desorption step

4.3.4. Competitive Biodegradation and Bioregeneration Studies

4.3.4.1. Bi-Solute Biodegradation of Phenol and 2-Chlorophenol. Bi-solute biodegradation studies showed that phenol was completely removed or converted into by-products within 48-72 hours of aeration in biodegradation reactors (Figures 4.30a-4.33a), as in the case of single-solute biodegradation in Section 4.1.3.1. Phenol profiles in batch biodegradation reactors followed a linear trend with respect to time during the first 24 hours of aeration (Figures 4.30a-4.33a) indicating that zero-order kinetics was applicable (R^2=0.9-0.94) for biodegradation (Schultz and Keinath, 1984). The zero-order biodegradation rate constants for phenol removal were determined for the first 24 hours of aeration when substrate concentration was sufficiently high. These were calculated to be between 5.87 and 9.20 mg phenol/L.h, where the S_0/X_0 ratios (initial substrate to microorganism ratio in batch

reactors) in RUNS 10-13 (Table 3.5) were between 0.11 to 0.14 mg phenol/mg MLSS. These zero-order rate constants obtained for phenol in the presence of 2-chlorophenol were higher than single-solute rate constants obtained in Section 4.1.3.1, which were between 4.88 and 6.36 mg phenol/L.h with S_0/X_0 ratios between 0.11 to 0.17 mg phenol/mg MLSS. Higher zero-order biodegradation rate constants for phenol in the bi-solute case can be attributed to the use of acclimated biomass. The presence of 2-chlorophenol did not cause inhibition in phenol biodegradation when acclimated biomass was used, as also evidenced by high OUR values, even in the first few hours of aeration (Figures 4.30b-4.33b). These high OURs decreased to below 5 mg O_2/L.h after 24-48 hours when phenol was depleted. On the other hand, when non-acclimated biomass was used in single-solute phenol biodegradation studies (Section 4.1.3), phenol caused substrate inhibition, particularly during the first few hours of aeration, as determined by low OUR values (Figures 4.7b-4.11b). Phenol is known to be self-inhibitory beyond a certain concentration according to Haldane kinetics (Goudar et al., 2000).

Biodegradation of 2-CP was very slow or even did not occur during the first 5 hours of aeration in RUNS 10-13 (Figures 4.30a-4.33a). Biodegradation of 2-CP occurred particularly after 24 hours of aeration when phenol concentrations decreased to very low levels, as seen in Figures 4.30a-4.33a. This is consistent with earlier findings that rapid chlorophenol transformation began only near exhaustion of phenol (Loh and Wang, 1998; Wang and Loh, 2000). Microorganisms apparently preferred the more biodegradable substance phenol instead of 2-CP. Also, in the study of Wang and Loh (2000), microorganisms preferentially utilized phenol instead of another chlorophenol 4-CP. Zero-order 2-CP biodegradation rate constants were determined for the first 48 hours of aeration with satisfactory high correlations (R^2=0.89-1). These were calculated to be between 1.43 and 2.55 mg 2-CP/L.h, where the S_0/X_0 ratios were between 0.09 to 0.12 mg 2-CP/mg MLSS. 2-CP biodegradation was much slower than phenol biodegradation as determined by lower rates and as also seen in Figures 4.30a-4.33a. At the end of 10 days of aeration, 2-CP was totally removed or decreased to below 12 mg/L.

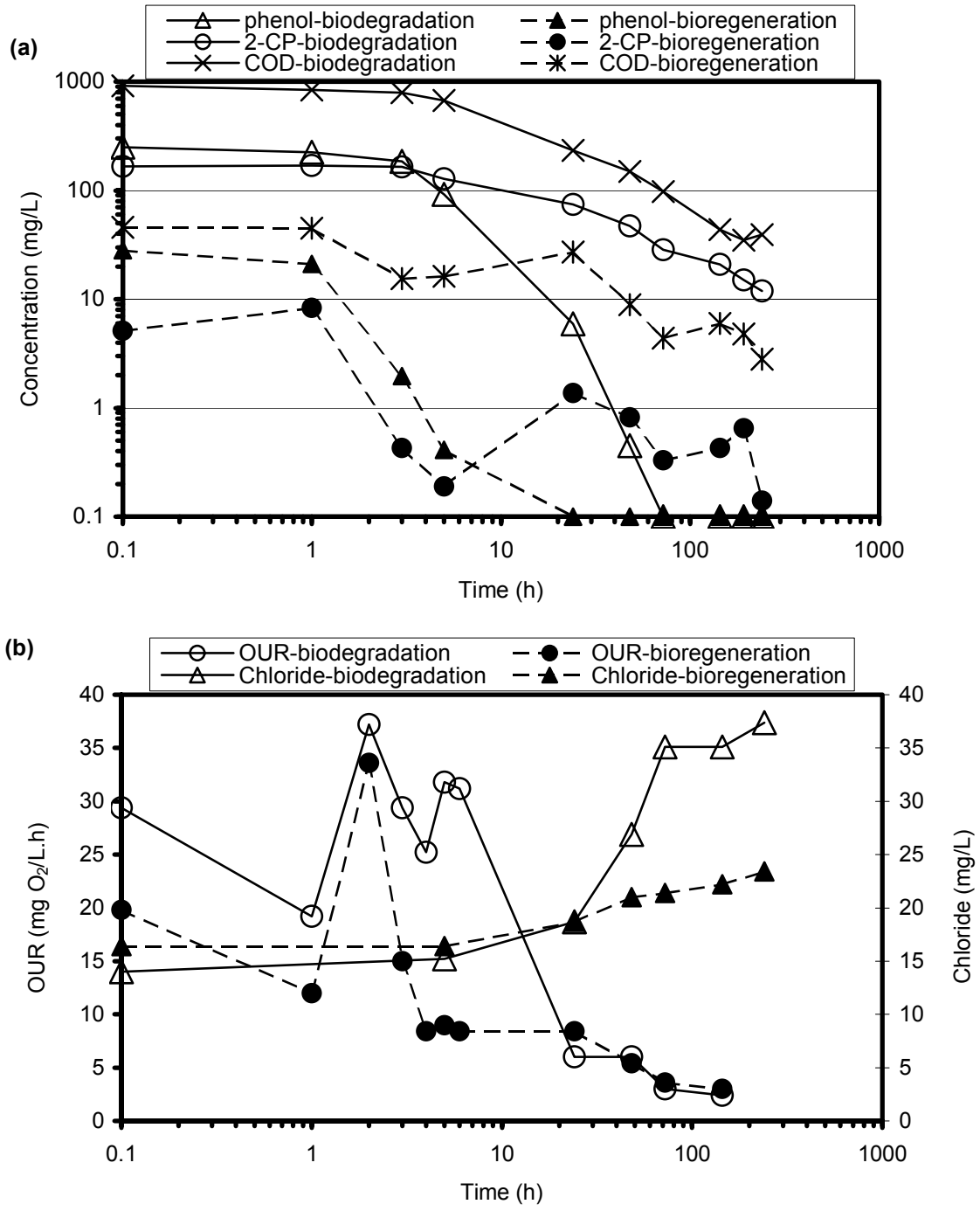


Figure 4.30. a) Phenol, 2-CP and COD profiles b) OUR and chloride profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L SA4 (powdered, thermally activated) in RUN 10.

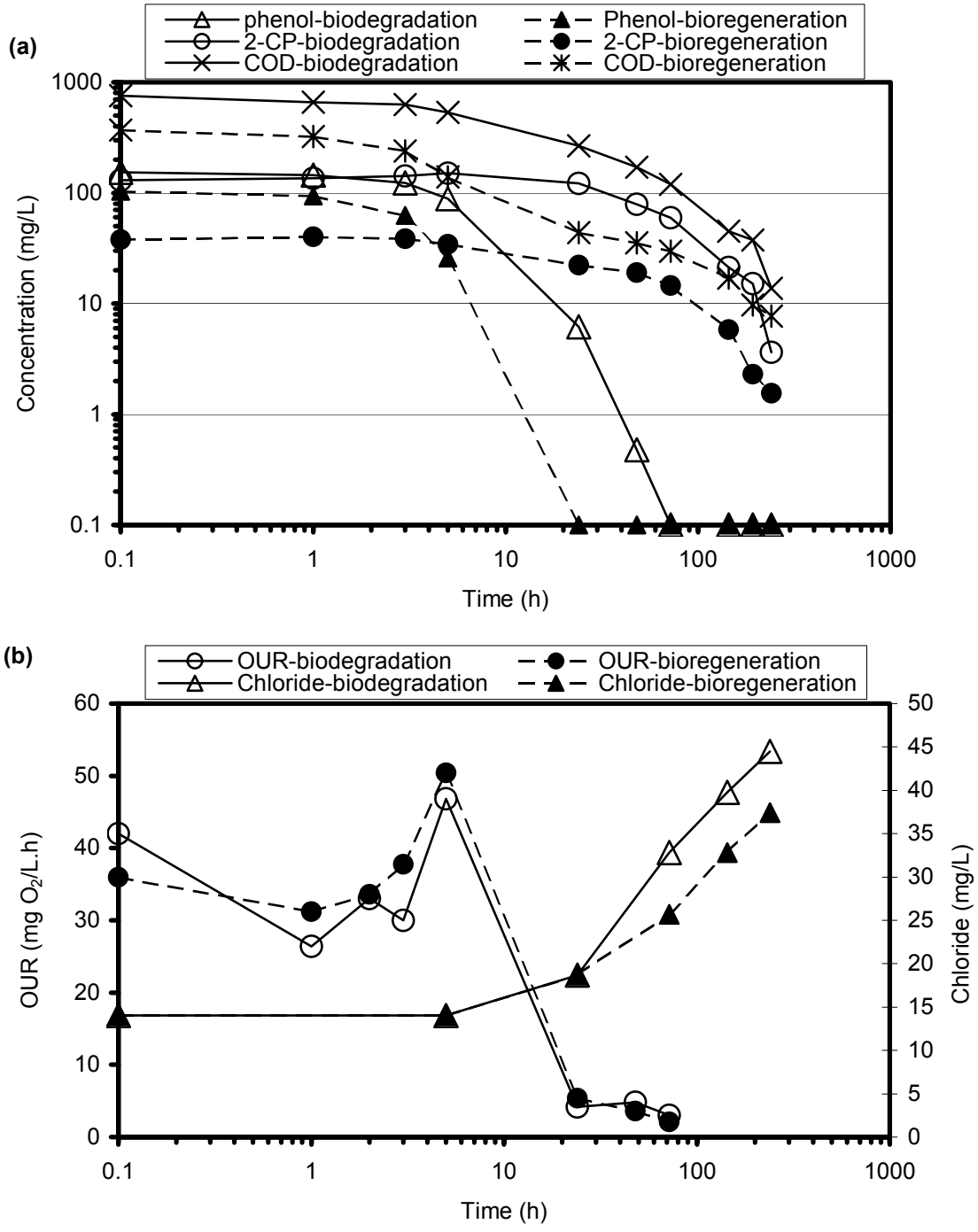


Figure 4.31. a) Phenol, 2-CP and COD profiles b) OUR and chloride profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L CA1 (powdered, chemically activated) in RUN 11.

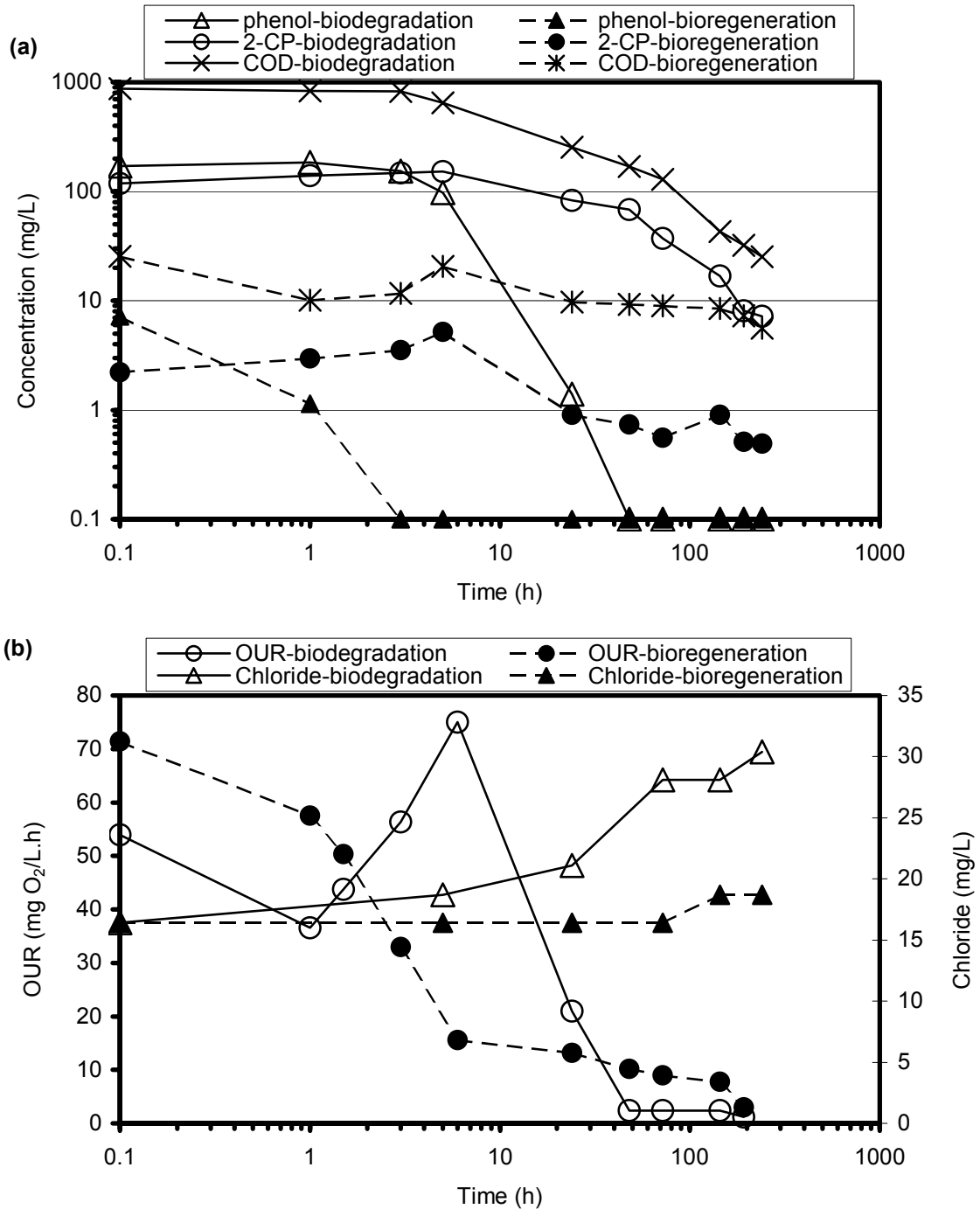


Figure 4.32. a) Phenol, 2-CP and COD profiles b) OUR and chloride profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L PKDA (granular, thermally activated) in RUN 12.

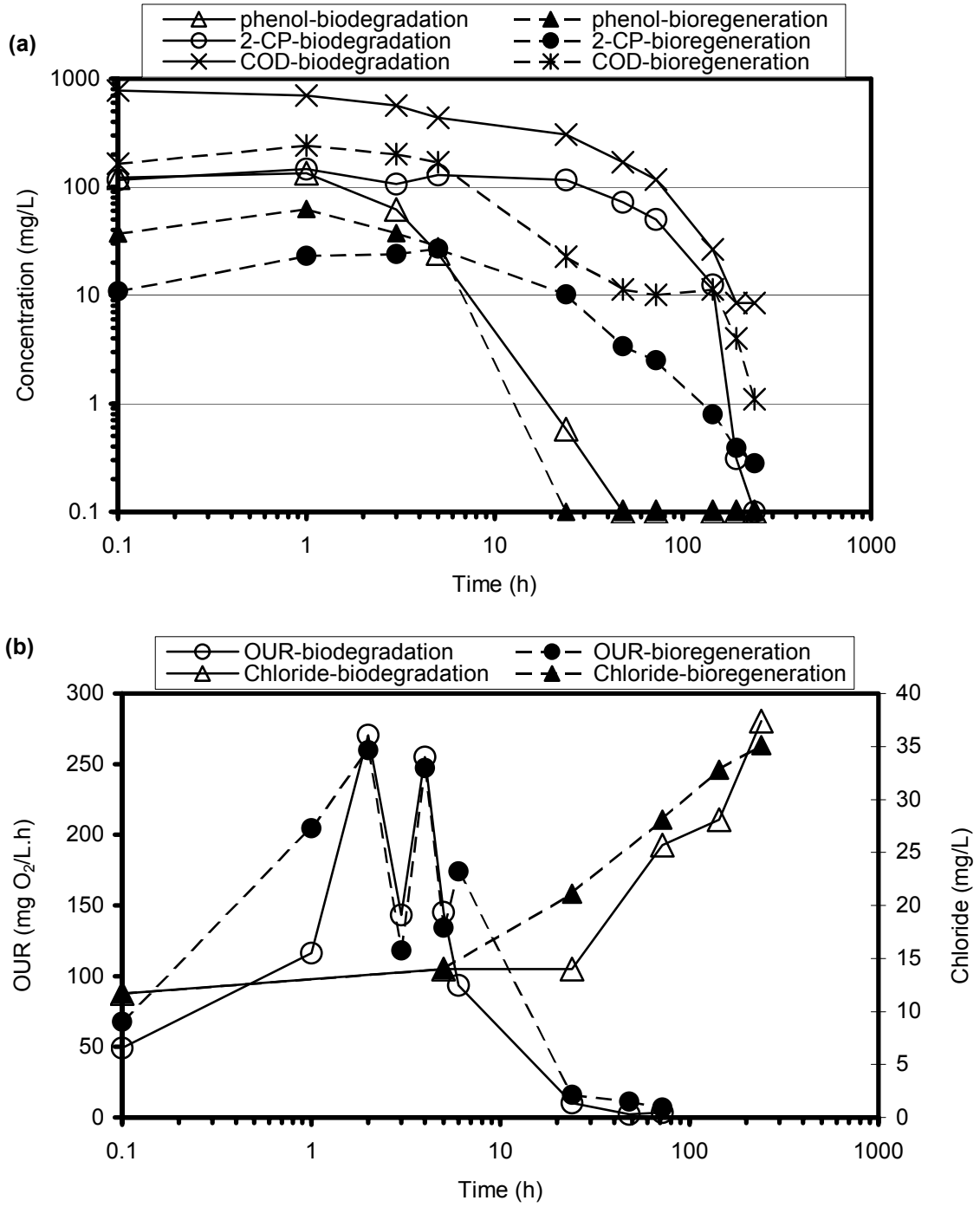


Figure 4.33. a) Phenol, 2-CP and COD profiles b) OUR and chloride profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L CAgran (granular, chemically activated) in RUN 13.

In the case of single-solute biodegradation in Section 4.2.3, 2-CP was not biodegraded with the acclimated biomass. This showed that 2-CP was only removed by a cometabolic pathway in the presence of phenol. In addition, the acclimation procedure reported in Section 4.5.3 resulted in domination of phenol-oxidizing bacteria. Phenol is a good growth substrate for cometabolism of chlorophenols because of structural analogy. Phenol-oxidizers easily induced the non-specific enzyme phenol monooxygenase, which was necessary for degradation of phenol as well as cometabolic transformation of 2-CP, as also shown in another study (Wang and Loh, 2000). In addition, phenol metabolism can also efficiently regenerate the consumed NAD(P)H, which is a necessary cofactor for the function of the monooxygenase enzyme (Saez and Rittmann, 1991). Therefore, 2-CP removal and also chloride formation continued even after depletion of phenol. Non-specific enzymes produced by microorganisms for phenol biodegradation could have continued their function with biodegradation of 2-CP even after depletion of phenol. This situation encountered in cometabolism is called the resting cell condition (Kocamemi, 2005).

Chloride ion concentrations were also measured throughout the batches (Figures 4.30b-4.33b) to determine dechlorination and/or mineralization of 2-CP. Chloride production at the end of 10 days was about 23-30 mg/L, which showed dechlorination of about 85-110 mg/L 2-CP. About 64-73 % of the initial 2-CP in biodegradation reactors was definitely dechlorinated. A sharp increase in chloride concentrations can be seen after 24 hours of aeration in Figures 4.30b-4.33b. This shows that the increased 2-CP removal after 24 hours was also accompanied by dechlorination.

COD removal also followed a zero-order biodegradation kinetics (R^2=0.94-0.98) during the first 24 hours of aeration. Zero-order biodegradation rate constants in this time period were between 18.24 and 26.94 mg COD/L.h, where the S_0/X_0 ratios were between 0.48 to 0.66 mg COD/mg MLSS. At the end of aeration, about 10-40 mg/L COD remained in the biodegradation reactors (Figures 4.30a-4.33a). Only about 10-20 mg/L of this residual COD was not caused by the remaining 2-CP, but stemmed from side-products of phenol and 2-CP removal or soluble microbial products (SMP). However, in the case of single-solute phenol biodegradation in Section 4.1.3.1, 30-50 mg/L non-phenolic COD was remaining at the end of aeration. The lower residual COD in bi-solute biodegradation

indicated that acclimated biomass produced lower SMP or by-products. Also, no other phenolic by-products were produced, as determined by the GC method applied (Section 3.7), which is specific for phenolic compounds.

4.3.4.2. Removal of Phenol and 2-Chlorophenol in Bioregeneration Reactors. In bioregeneration reactors operated in parallel to biodegradation reactors, phenol and 2-CP concentrations in the bulk liquid were always much lower (Figures 4.30a-4.33a). This was because not all of the phenol and 2-CP were desorbed, but only a quotient of them were desorbed into the bulk, as discussed in Section 4.3.3. RUNS 14 and 15 (Table 3.5) present a comparison between bioregeneration of thermally (SA4 and PKDA) and chemically activated (CA1 and CAgran) carbons (Figures 4.34, 4.35). Phenol and 2-CP concentrations in the bulk liquid were always much lower in the bioregeneration reactors containing thermally activated carbons (Figures 4.34a, 4.35a), in relation to the much lower desorption efficiencies for these carbons (Table 4.14).

In the bulk liquid of bioregeneration reactors with SA4 in RUNS 10 and 14, the initial phenol and 2-CP concentrations during the first hour of aeration were about 28-45 mg/L and 5-8 mg/L, respectively (Figures 4.30a, 4.34a). However, in bioregeneration reactors with PKDA in RUNS 12 and 15, concentrations of phenol and 2-CP during the first hour were about 7-9 mg/L and 2-3 mg/L, respectively (Figures 4.32a, 4.35a). The reason for higher initial bulk concentrations in the case of the powdered activated carbon SA4 was the faster desorption from powdered carbons compared with the granular ones as discussed in Sections 4.1.2 and 4.2.2. This was attributed to the effect of particle size. Since GAC particles are much larger than PAC particles, diffusive transport from GAC becomes slower.

Phenol concentrations decreased below 1 mg/L during the first 5 hours of aeration in all bioregeneration reactors with the thermally activated carbons SA4 and PKDA (Figures 4.30a, 4.32a, 4.34a, 4.35a). Considering Figure 4.5 showing desorption of phenol with respect to time in Section 4.1.2, desorption of phenol was expected to occur continuously during the first few hours, particularly from the bioregeneration reactors containing granular carbon PKDA. However, phenol concentrations continuously decreased even in reactors containing PKDA. Obviously, the biodegradation rate was higher than the

desorption rate of phenol. On the other hand, 2-CP concentrations increased to 4-5 mg/L during the first 5 hours in reactors with PKDA, showing that the biodegradation rate of 2-CP was lower than the desorption rate. In all reactors with SA4 or PKDA, 2-CP was depleted or decreased to below 1 mg/L at the end of 24 hours of aeration (Figures 4.30a, 4.32a, 4.34a, 4.35a).

In bioregeneration reactors of RUNS 11 and 14 with CA1, initial phenol and 2-CP concentrations during the first hour of aeration were about 90-100 mg/L and 30-40 mg/L, respectively (Figures 4.31a, 4.34a). Phenol concentrations decreased rapidly until the fifth hour down to 25-45 mg/L, and phenol was depleted or decreased below 2 mg/L at the end of 24 hours. In bioregeneration reactors of RUNS 13 and 15 with CAgran, the initial phenol and 2-CP concentrations during the first hour of aeration were about 40-60 mg/L and 10-20 mg/L, respectively (Figures 4.33a, 4.35a). These initial concentrations were much lower compared with the powdered carbon CA1, because of the slower diffusivity from the granular one CAgran. Phenol concentrations decreased rapidly until the fifth hour down to 20-30 mg/L, and phenol was depleted at the end of 24 hours, also in case of CAgran. Although desorbability of phenol was much higher from chemically activated carbons compared with thermally activated ones, biodegradation rate of phenol exceeded desorption rate also for chemically activated carbons.

On the other hand, 2-CP concentrations were almost constant during the first 5 hours, decreased to 10-20 mg/L at the end of 24 hours and depleted or decreased to below 2 mg/L at the end of 3-8 days of aeration in bioregeneration reactors with CA1 (Figures 4.31a, 4.34a). However, in bioregeneration reactors with CAgran (Figures 4.33a, 4.35a), 2-CP concentrations increased during the first 5 hours, decreased to about 10 mg/L at the end of 24 hours and decreased to below 1 mg/L at the end of 3-6 days of aeration. Increased 2-CP concentrations during the first hours showed that desorption rate of 2-CP exceeded biodegradation rate of 2-CP, also in case of the granular carbon CAgran. However, in case of CA1, these rates were similar resulting in a constant 2-CP profile during the first few hours.

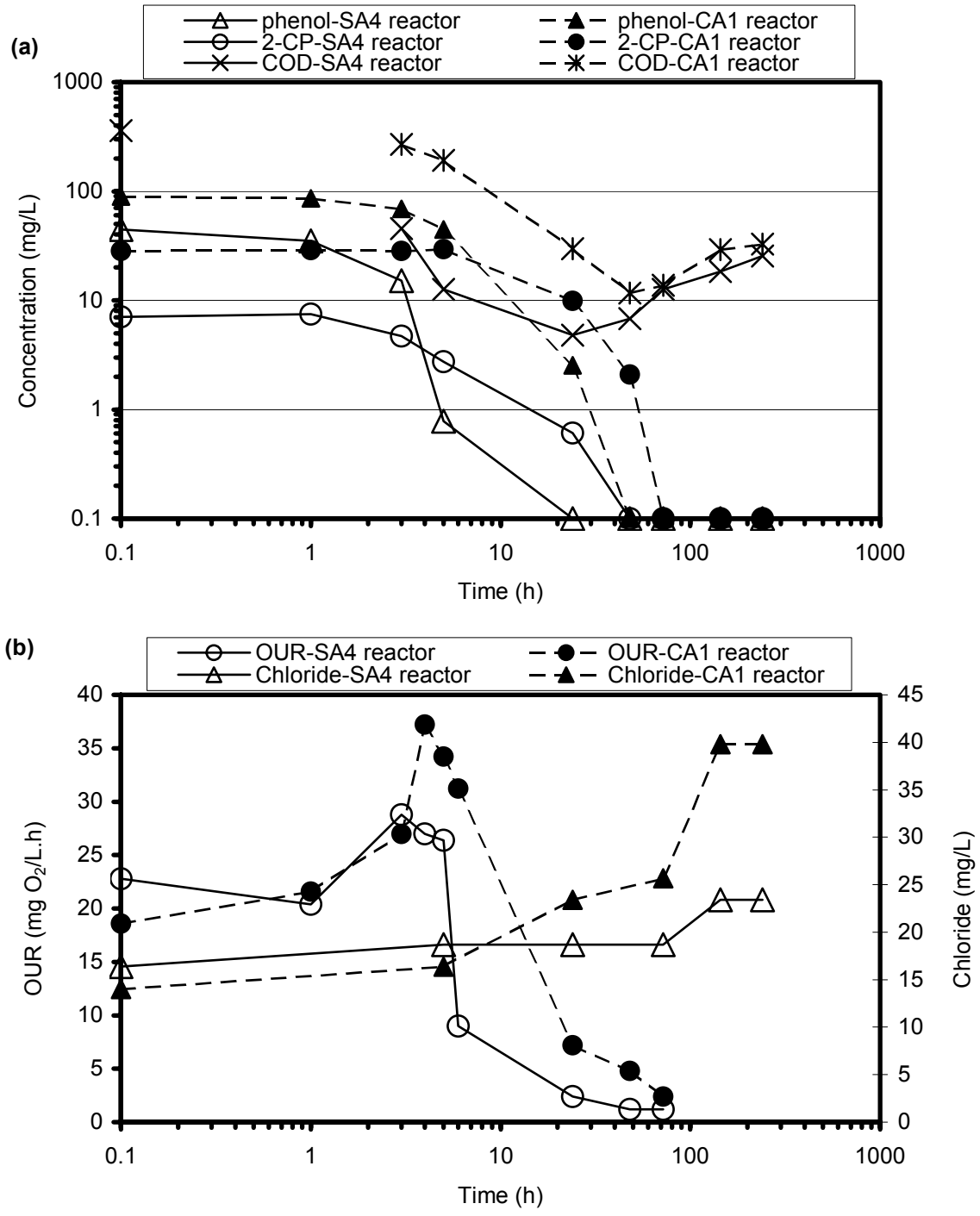


Figure 4.34. a) Phenol, 2-CP and COD profiles b) OUR and chloride profiles in bioregeneration reactors with SA4 (powdered, thermally activated) and CA1 (powdered, chemically activated) in RUN 14.

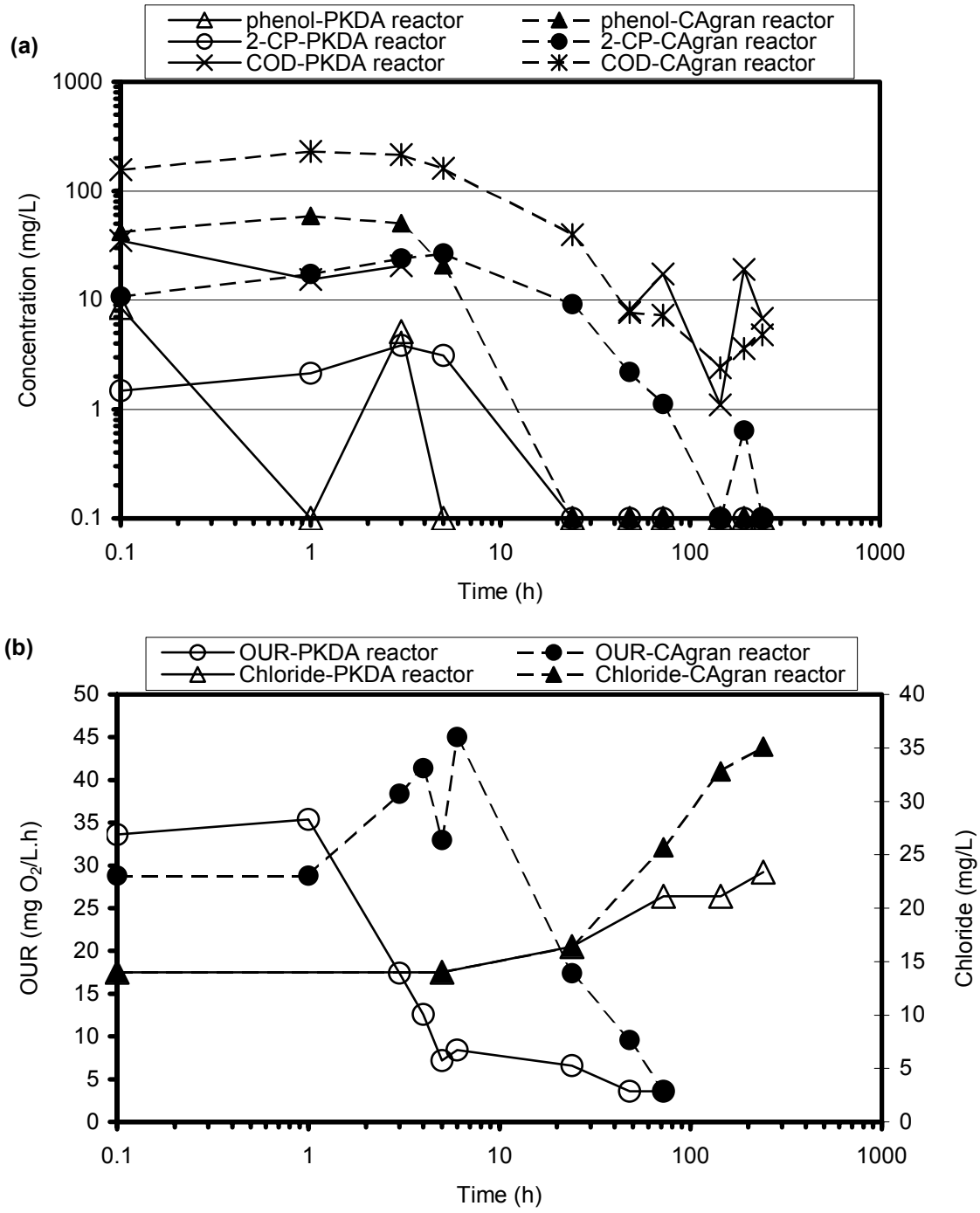


Figure 4.35. a) Phenol, 2-CP and COD profiles b) OUR and chloride profiles in bioregeneration reactors with PKDA (granular, thermally activated) and CAgran (granular, chemically activated) in RUN 15.

In most of the bioregeneration runs, COD concentrations decreased to below 10 mg/L at the end of 48 hours (Figures 4.30a-4.35a). Non-phenolic COD was below 5 mg/L at the end of 10 days of aeration in all runs except for RUN 14. In this run residual COD concentrations increased from 7 and 12 mg/L at 48 hours to 26 and 33 mg/L at 240 hours in SA4 and CA1 reactors, respectively (Figure 4.34a). This apparent increase in COD in this run was probably due to production of SMP as a result of lysis of dead microbial cells.

4.3.4.3. Investigation of Bioregeneration by OURs and Chloride Formation. In bioregeneration reactors with thermally activated carbons, oxygen uptake rates (OURs) were lower during the first 5-6 hours of aeration compared with the parallel biodegradation reactors (Figure 4.30b, 4.32b), except for the first hour in RUN 12 with PKDA. After 24 hours of aeration, OURs in SA4 and PKDA reactors exceeded or equalled the ones in biodegradation reactors. However, in bioregeneration reactors with chemically activated carbons, OURs were higher than or comparable with OURs in parallel biodegradation reactors (Figure 4.31b, 4.33b). Also, OURs in bioregeneration reactors with the chemically activated carbons CA1 and CAgran exceeded those in parallel reactors with thermally activated carbons SA4 and PKDA (Figure 4.34b, 4.35b). After depletion of bulk phenol, OURs decreased to below 10 mg O_2/L.h in all bioregeneration reactors. This showed that the more readily biodegradable compound, phenol was mainly responsible for high OURs. Low OURs were observed in reactors containing thermally activated carbons because of lower bulk phenol concentrations. However, in reactors containing chemically activated carbons, desorption of phenol and therefore bioregeneration occurred more than the thermally activated ones, as also determined by higher OURs in these reactors.

The less biodegradable component, 2-CP did not contribute much to the OURs because of its much lower biodegradation rate (Section 4.3.4.1) and much lower desorbability (Section 4.3.3). Although phenol and 2-CP concentrations were always much lower in all bioregeneration reactors in comparison with biodegradation reactors (Figures 4.30a-4.33a), OURs in bioregeneration reactors were not that much lower or sometimes they were even equal to or greater than OURs in biodegradation reactors (Figures 4.30b-4.33b). The reason for this was the continuous bioregeneration of activated carbons resulting in low concentrations in the bulk with comparatively very high corresponding OURs in bioregeneration reactors. Also, inhibition due to higher bulk 2-CP concentrations

in biodegradation reactors might be another reason. Oxidating coupling reactions might have also contributed to high OURs in bioregeneration reactors as also discussed in Section 4.1.3.2.

Chloride formations in bioregeneration reactors were used as determinants for bioregeneration of activated carbons for adsorbed 2-CP. As expected from the high bulk 2-CP concentrations in reactors with chemically activated carbons (Figures 4.34a, 4.35a), chloride concentrations were higher in reactors containing these carbons compared with thermally activated carbons (Figures 4.34b-4.35b). In all runs, chloride formation did not occur much during the first 24 hours and occurred after 72 hours, since biodegradation of 2-CP took place after depletion of phenol.

In bioregeneration reactors with thermally activated carbons, chloride productions were between 2.3-9.4 mg/L, theoretically corresponding up to 34 mg/L 2-CP. Such a high 2-CP concentration was not observed in the bulk liquid, because desorbed 2-CP was continuously biodegraded resulting in a continuous bioregeneration. These chloride productions equalled to a mineralization of 16.6-68 mg 2-CP in these reactors. These amounts of mineralized 2-CP in bioregeneration reactors should be considered as the amount of 2-CP removed from the loading on carbon. Hence, considering only 2-CP loadings on carbons (Table 3.5), bioregeneration of SA4 was calculated to be about 13 % in RUNs 10 and 14. Bioregeneration of PKDA was calculated to be about 4.5 % and 18 % for 2-CP in RUNs 12 and 15, respectively.

On the other hand, chloride productions were between 21.1-25.8 mg/L in bioregeneration reactors with chemically activated carbons. These theoretically correspond to 2-CP concentrations of 76.4-93.4 mg/L, which were never actually achieved in the bulk. Therefore, this showed the presence of a continuous bioregeneration of 2-CP loaded activated carbons. The chloride productions corresponded to a mineralization of 152.8-186.8 mg 2-CP in these reactors. Hence, bioregeneration of CA1 was calculated to be about 44.3 % and 48.8 % for 2-CP in RUNs 11 and 14, respectively. Bioregeneration of CAgran was calculated to be about 51.8 % and 43.4 % for 2-CP in RUNs 13 and 15, respectively. These bioregeneration efficiencies are actually the minimum quantifiable efficiencies. Additionally, biosorption of 2-chlorophenol might be also possible.

Bioregeneration of chemically activated carbons were higher in correspondence with the higher desorbabilities of 2-CP from these carbons (Table 4.14).

4.3.4.4. Quantification of Bioregeneration. Post-bioregeneration adsorption experiments (Section 3.5.3) made direct measurement of bioregeneration possible by comparing adsorption capacities before and after bioregeneration (Eq. 2.7 in Section 2.3.6.2). The presence of very low 2-CP concentrations in the bulk and the high biodegradation rate of phenol did not allow the use of the Freundlich analogy for quantification of bioregeneration in these bi-solute studies. The direct measurement method resulted in bioregeneration efficiencies at the end of 10 days of aeration as tabulated in Table 4.15, except for RUN 15, where no post-bioregeneration adsorption was applied. Initial conditions in these bioregeneration runs can be seen in Table 3.5.

Table 4.15. Bioregeneration rates and efficiencies for activated carbons competitively loaded with phenol and 2-chlorophenol.

Run No	Carbon type	Bioregen. Efficiency for Phenol loading (%)	Bioregen. Efficiency for 2-CP loading (%)	Bioregen. rate constant for Phenol loading, k (h^{-1})	Bioregen. rate constant for 2-CP loading, k (h^{-1})
10	SA4	23.7	39.4	0.1645	n.a.
11	CA1	65.2	66.3	0.1330	0.0055
12	PKDA	50.6	38.6	n.a.	n.a.
13	CAgran	81.5	52.1	n.a.	0.0105
14	SA4	27.0	28.9	0.1443	0.0198
14	CA1	55.4	66.6	0.0731	0.0227
15	PKDA	n.a.	n.a.	n.a.	n.a.
15	CAgran	n.a.	n.a.	n.a.	0.0106

n.a.: Rate constants were not applicable in some cases because of low correlation (R^2<0.75).

Bioregeneration efficiencies were higher both for phenol and 2-CP loadings in the case of chemically activated carbons compared with thermally activated carbons (Table 4.15), as in the previous single-solute studies with phenol. This was also in accordance with higher desorbabilities (Table 4.14) of two competing compounds from the chemically activated carbons. However, bioregeneration efficiencies calculated for these carbons were lower than the desorbabilities, particularly for phenol. This indicated that bioregeneration efficiencies tabulated in Table 4.15 are lower than the actual bioregeneration efficiencies. Possibly, obstruction of activated carbon pores by the presence of biomass slime matrix

and adsorption of some soluble microbial products during bioregeneration process resulted in decreased adsorption capacities in post-bioregeneration adsorption.

In the case of thermally activated carbons SA4 and PKDA, the average bioregeneration of the total phenol and 2-CP loadings was about 28-30 % for SA4 in RUNs 10 and 14, and about 44 % for PKDA in RUN 12. However, desorbability of phenol was about 32-33 %, and that of 2-CP was only about 6-8 % (Table 4.14) from these carbons. It should also be considered that these bioregeneration efficiencies in Table 4.15 (and those average efficiencies given above) can be lower than actual efficiencies. Hence, it can be deduced that considering both phenol and 2-CP, bioregeneration efficiencies were higher than desorbabilities of phenol and 2-CP in case of thermally activated carbons. In Section 4.1.3.5, this observance was attributed to the activity of extracellular enzymes when phenol was the only carbon source. The exoenzymes seem to be effective on bioregeneration of thermally activated carbons, rather than chemically activated carbons, also in the case of bi-solute bioregeneration when an acclimated biomass was used.

First-order bioregeneration rate constants were calculated both for phenol and 2-CP with high correlations (R^2=0.90-1) as explained in Section 4.1.3.3. Bioregeneration rate constants for phenol were much higher compared with those for 2-CP (Table 4.15). This was due to higher desorbability and higher biodegradation rate of phenol. Bioregeneration rate constants for phenol in bi-solute case (Table 4.15) were also much higher compared with those in single-solute case (Table 4.5). This can be also attributed to higher desorbability (Section 4.3.3) and higher biodegradation rates of phenol (Section 4.3.4.1) in bi-solute case compared with single-solute case.

For comparison purposes, first-order biodegradation rate constants were also calculated both for phenol and 2-CP considering the total aeration period during bi-solute biodegradation in RUNS 10-13. Phenol biodegradation rate constants were between 0.1331-0.2237 h^{-1} (R^2=0.99-1) and 2-CP biodegradation rate constants were between 0.0102-0.0163 h^{-1} (R^2=0.88-0.97). These biodegradation rates were not apparently higher compared to bioregeneration rates (Table 4.15), contrary to the earlier findings for phenol as a single-solute (Section 4.1.3.4). When, phenol was the only substrate, desorption was a limiting factor for bioregeneration rate. However, in the bi-solute case the comparable rates

for biodegradation and bioregeneration suggested that biodegradation was the rate-limiting factor for bioregeneration. In the bi-solute case, phenol desorption was much easier compared with the single-solute case. This was because phenol was adsorbed on less energetic adsorption sites than 2-CP in the bi-solute case. Therefore, desorption did not become a rate-limiting factor for phenol. On the other hand, biodegradation of 2-CP was very slow, so that biodegradation was rate-limiting for bioregeneration rather than desorption. Also, the first-order COD biodegradation rate constants were calculated to be between 0.0133-0.0192 h^{-1} (R^2=0.88-0.97) considering the total aeration period of 10 days. These were closer to 2-CP biodegradation rates because phenol biodegradation ceased at the end of 24-48 hours, and hence a great portion of COD consisted of 2-CP.

At the end of bioregeneration runs, activated carbon samples were taken for solvent extraction (Section 3.5.3) in order to determine the extractable phenol and 2-CP loadings remaining on activated carbons. Extracted phenol and 2-CP loadings were proportioned to the initial loadings (Q_1) in the bioregeneration runs. No phenol was extracted from the CA1 carbon samples in RUNS 11 and 14, and only 0.03 % of initial phenol loading (Q_1) was extracted from CAgran in RUN 15. On the other hand, only 0.56 % and 0.35 % of initial 2-CP loadings were extracted from CA1 reactors in RUNS 11 and 14, respectively. In RUN 15, only 2.22 % of initial 2-CP was extracted from CAgran. This showed that almost all of the extractable phenol was removed during bioregeneration of chemically activated carbons. Considering the bioregeneration efficiencies exceeding 50 % for chemically activated carbons (Table 4.15), solvent extraction resulted in a desorption of maximum 2.22 % of initial 2-CP. Most of the extractable 2-CP was also removed during bioregeneration, and further solvent extraction did not improve desorbability.

In the case of thermally activated carbons, extraction was more efficient. About 0.22 % and 1.12 % of initial phenol loadings were extracted at the end of RUN 14 with SA4 and RUN 15 with PKDA, respectively. On the other hand, 3.19 % and 9.45 % of initial 2-CP loadings were extracted at the end of RUN 14 with SA4 and RUN 15 with PKDA, respectively. Higher extraction efficiencies for 2-CP was because 2-CP was adsorbed with higher adsorption energies and hence bioregeneration process could not remove all of the 2-CP. Although, exoenzymatic bioregeneration seemed to increase the total bioregeneration efficiency, there was still some phenol and 2-CP on activated carbon,

where enzymes could not reach or could not react with. This finding is in agreement with the previous discussion in Section 4.1.3.5 suggesting that enzymatic activity was most probable in mesopores, but not very much probable in micropores. When these extraction efficiencies of less than 10 % of the initial loadings are combined with the bioregeneration efficiencies, there is a great portion of non-extractable phenol and 2-CP. This non-extractable portion was due to chemical adsorption. It can be deduced that the use of solvent extraction for quantification of bioregeneration (Section 2.3.6.3) will not be a correct way of measuring bioregeneration when chemisorption occurs. Considering the lesser bioregeneration and desorption efficiencies for thermally activated carbons, these solvent extraction studies showed that chemical adsorption was more plausible on thermally activated carbons compared with chemically activated ones.

4.3.4.5. Investigation by Environmental Scanning Electron Microscopy. Microorganisms attached on the activated carbon surfaces were observed by Environmental Scanning Electron Microscopy (ESEM). Activated carbon samples taken after 3 days of aeration from each bioregeneration reactor in RUNS 14 and 15 were analyzed by ESEM as explained in Section 3.7. Figures 4.36-4.39 illustrate samples of ESEM images of microorganisms on each type of activated carbon. Other ESEM images can be seen in Figures B.4-B.8 in Appendix B. As discussed in Section 4.1.3.7, attachment of microorganisms on the surface of activated carbon may be important in terms of bioregeneration. The attached microorganisms might contribute to bioregeneration either by creating a concentration gradient or by excreting extracellular enzymes through the activated carbon pores.

ESEM analyses showed that groups of cocci-shaped bacteria with diameters of 1-2 μm dominated the microflora. Considering that the activated sludge used in bioregeneration studies had been acclimated to phenol and 2-CP for more than a year, these dominating bacteria can be considered as phenol-oxidizers. These cocci-shaped bacteria attached to the outer surface or internal cavities of activated carbon, usually in groups like a bunch of grapes (Figures 4.36-4.39). On the other hand, larger protozoan-like microorganisms and filamentous bacteria were less encountered.

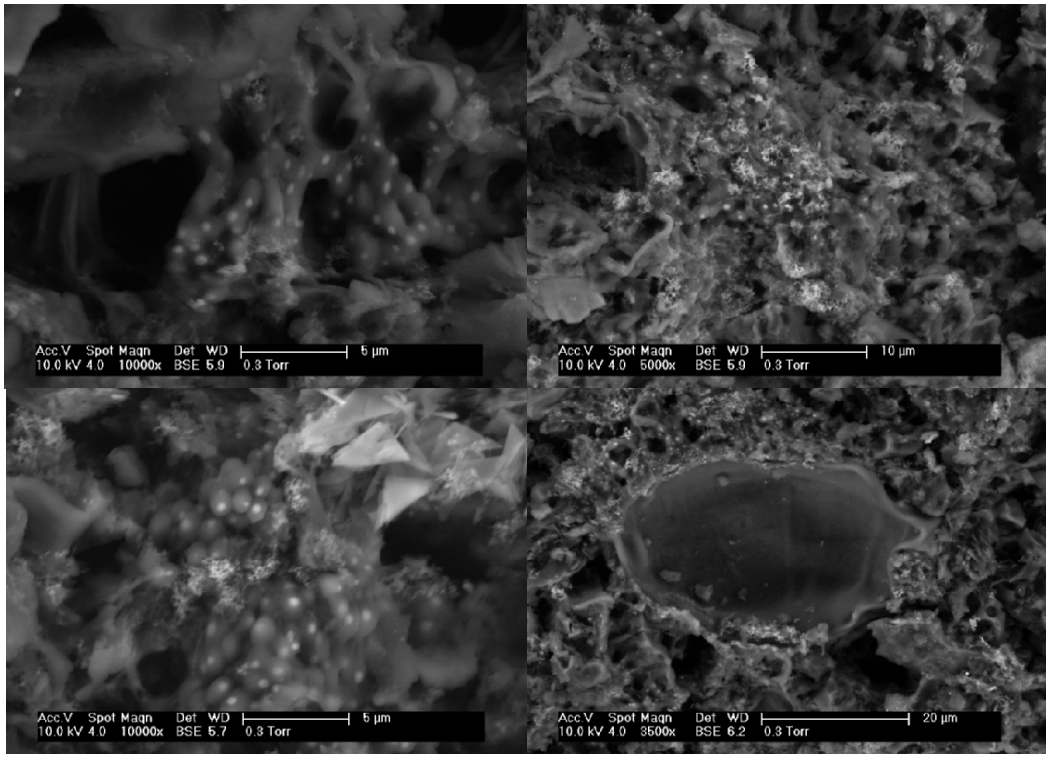


Figure 4.36 Scanning Electron Micrographs of microorganisms on SA4 in RUN 14.

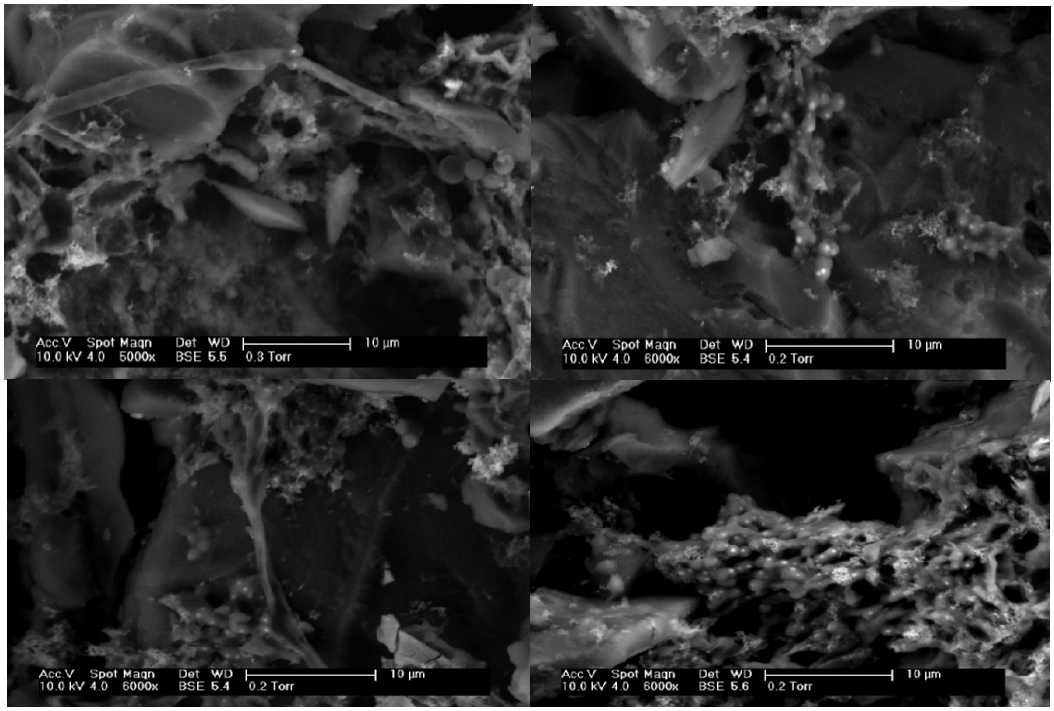


Figure 4.37 Scanning Electron Micrographs of microorganisms on CA1 in RUN 14.

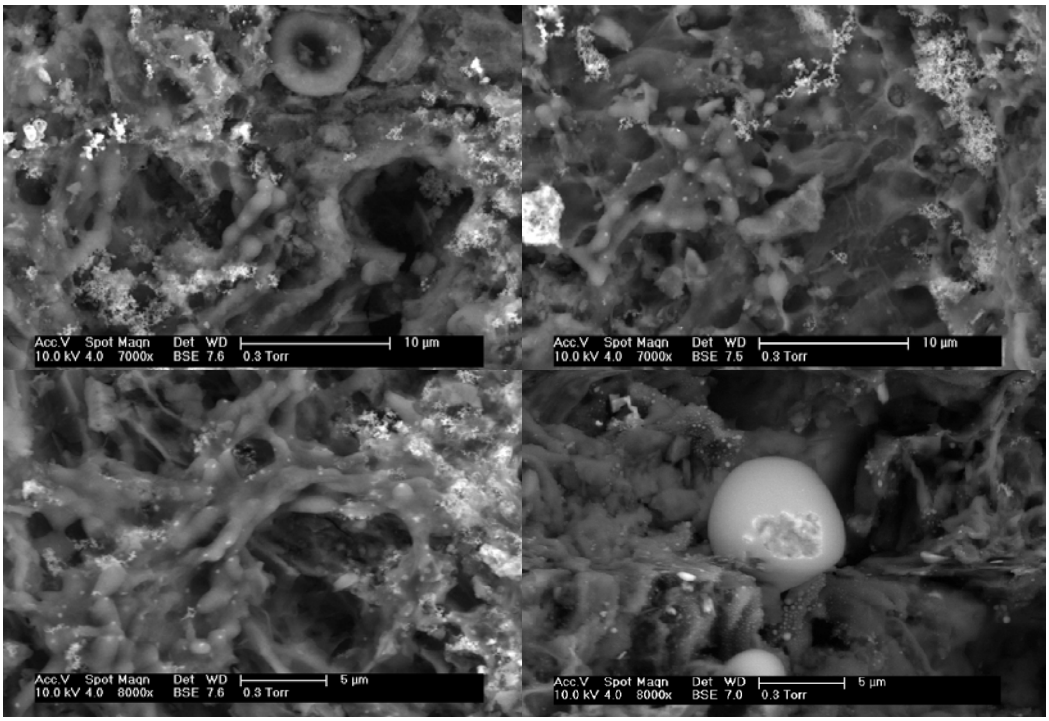


Figure 4.38. Scanning Electron Micrographs of microorganisms on PKDA in RUN 15

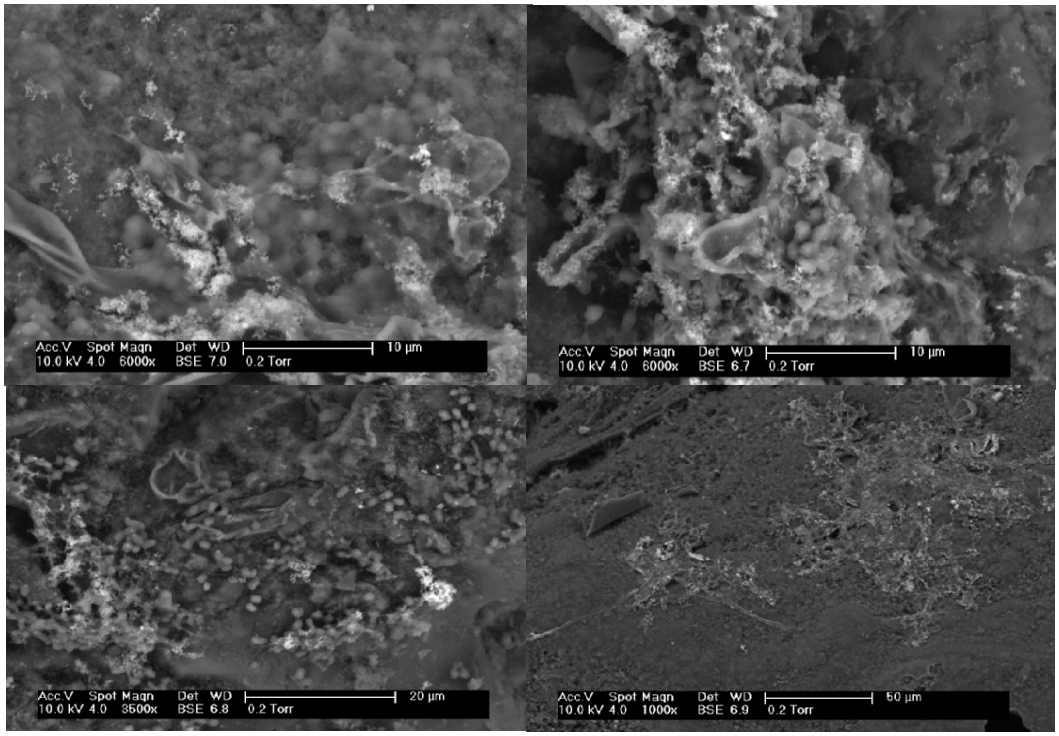


Figure 4.39. Scanning Electron Micrographs of microorganisms on CAgran in RUN 15.

Analyses of CAgran samples taken after 10 days of aeration (Figure B.8) showed that phenol oxidizers decreased in number, but filamentous bacteria and density of slime matrix increased with time. Larger particles of powdered carbon CA1 (Figure 4.37) compared with SA4 (Figure 4.36) enabled better attachment for the groups of cocci-shaped bacteria. The groups of bacteria also attached to the carbon surface together with the polysaccaride slime matrix, as better observed for large GAC particles in Figures 4.38 and 4.39. The slime matrix on carbon surfaces was much less dense compared with the slime matrix of non-acclimated biomass in Section 4.1.3.7. This showed that less cellular debris was produced, which indicated a lesser growth for phenol-oxidizers.

4.4. Experiments with 2-Nitrophenol as a Model Compound

In the last part of experimental work, 2-nitrophenol (2-NP) was used as the model compound in order to investigate the effect of substitution of the nitro (NO_2) group to the benzene ring of phenol. 2-NP is also known to be adsorbable on activated carbon, but less biodegradable than phenol as 2-CP. The differences in adsorbabilities, desorbabilities and biodegadabilities of 2-CP and 2-NP will enable to investigate the effect of substitution to phenol on bioregeneration of activated carbon. Only powdered carbons SA4 and CA1 were used in studies with 2-NP, because previous studies with phenol and 2-CP showed that powdered and granular countertypes did not differ much, except in adsorption and desorption rates.

4.4.1. Adsorption Studies with 2-Nitrophenol

The Equilibrium time for adsorption studies was determined as 4 days for SA4, and 7 days for CA1 although most of 2-NP adsorption took place in the first 5 hours as seen in Figure 4.40. The adsorption isotherm data for 2-NP are plotted in Figure 4.41. The adsorption isotherm data fitted the Freundlich equation (Eq. 4.1), as in the previous adsorption isotherms with phenol and 2-CP. Freundlich adsorption isotherm constants obtained by regression analysis for 2-NP are shown in Table 4.16.

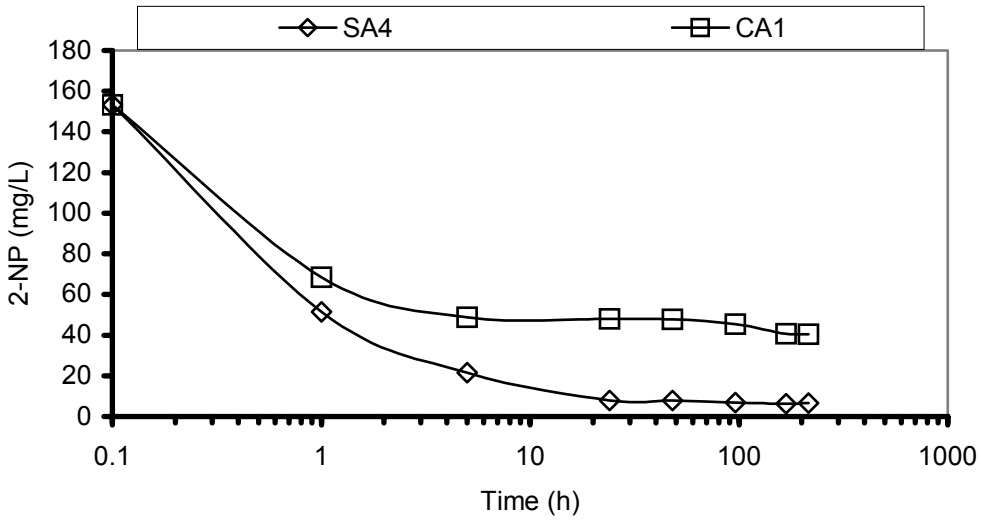


Figure 4.40. 2-Nitrophenol profiles during adsorption onto 1 g/L of activated carbons SA4 and CA1.

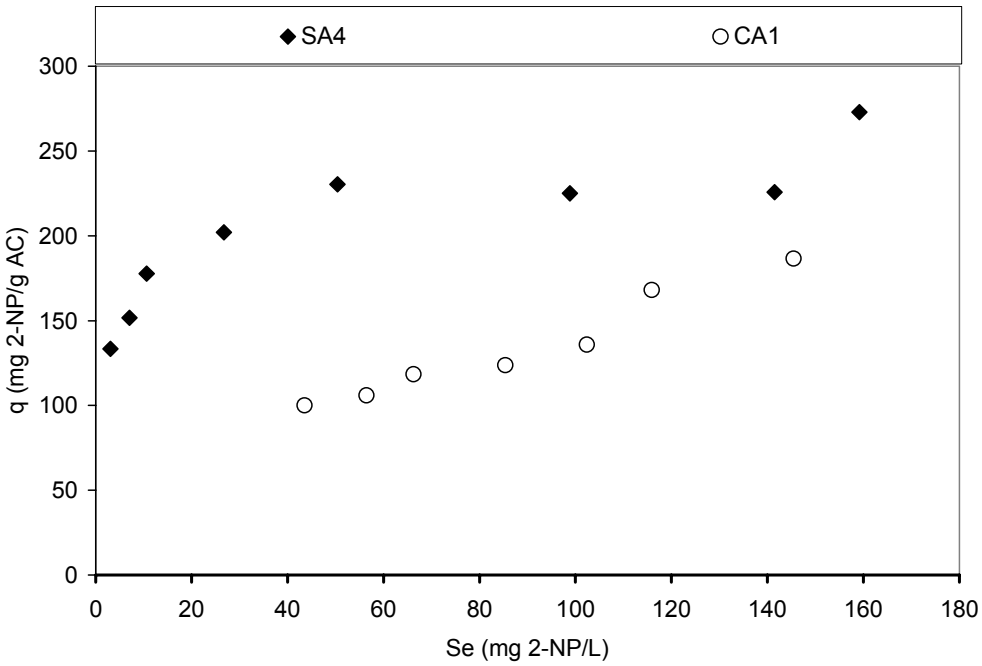


Figure 4.41. 2-Nitrophenol adsorption isotherms for activated carbon types SA4 and CA1.

Table 4.16. Freundlich isotherm constants for 2-nitrophenol adsorption.

Carbon type	**Physical form**	**Activation method**	**K [(mg/g) (L/mg)$^{1/n}$]**	**1/n**	**R^2**
Norit SA4	Powdered	Thermal	116.4	0.1552	0.92
Norit CA1	Powdered	Chemical	13.2	0.5223	0.92

Higher K and lower 1/n values (Table 4.6) obtained for SA4 and adsorption data in Figures 4.40 and 4.41 showed that the thermally activated carbon (SA4) was obviously a better adsorbent for 2-NP compared with the chemically activated one (CA1), as in the cases of phenol and 2-CP. The Freundlich constant K, which shows the adsorption capacity, was higher for 2-NP adsorption compared with the ones for phenol (Table 4.1) and 2-CP (Table 4.6) in the case of SA4. Also the 1/n value obtained for 2-NP was lower than those for phenol and 2-CP indicating a better adsorption intensity of 2-NP rather than phenol and 2-CP on the thermally activated carbon SA4.

The isotherm curves tended to reach saturation as the equilibrium concentrations increased (Figure 4.41). The theoretical Langmuir equation (Eq. 4.2) was also applied for the 2-NP adsorption isotherms with high correlations (Table 4.17). The R_L (dimensionless

separation factor) values for each activated carbon (Table 4.17) varied between 0 and 1 indicating a favorable adsorption of 2-NP on each carbon type. The adsorption of 2-NP on two thermally activated carbons was more favorable and closer to the irreversible adsorption edge of R_L=0 as evidenced from relatively lower R_L values. This finding is in agreement with the results of subsequent desorption studies.

Table 4.17. Langmuir isotherm constants for 2-nitrophenol adsorption.

Carbon type	Q° (mg/g)	b (L/mg)	R^2	R_L
Norit SA4	256.4	0.1703	0.98	0.108
Norit CA1	312.5	0.0092	0.84	0.653

4.4.2. Desorption Studies with 2-Nitrophenol

The desorption equilibrium time was determined as 24 hours for both carbon types SA4 and CA1. The Freundlich type isotherm equation (Eq. 4.1) was applied to desorption data (Figure 4.42) for both carbon types with satisfactory correlations. The desorption Freundlich isotherm constants are shown in Table 4.18. High K value for SA4 showed that 2-NP loading was still high after desorption. Also, the lower 1/n value in the case of SA4 indicated that desorption of 2-NP was more difficult from this carbon. These findings showed that the desorbability of 2-NP from thermally activated carbon SA4 was lower. Desorption capacities were higher for the chemically activated carbon CA1, as in previous phenol and 2-CP desorption studies (Section 4.1.2 and 4.2.2). These findings are also supported by the total desorption efficiencies of 2-NP for SA4 and CA1 carbons in Table 4.18.

Reversibility of adsorption was higher in the case of thermally activated SA4 for 2-nitrophenol (about 35 % in Table 4.18) compared with 2-chlorophenol (about 13 % in Table 4.9) and phenol (about 20 % in Table 4.4). However, in the case of chemically activated carbon CA1, reversibility of adsorption for 2-NP (about 69 % in Table 4.18) was close to that for 2-CP (66 % in Table 4.9) and less than that for phenol (about 87 % in Table 4.4). This finding with CA1 is in agreement with the statement that ortho-substituted phenols are more irreversibly adsorbed compared with phenol (Yonge et al., 1985). However, a contrary result was obtained in the case of thermally activated carbon SA4.

This finding is in agreement with the statement that nitrophenols are not subject to oxidative coupling reactions (Vidic et al., 1993). Hence, oxidative coupling reactions seemed to be effective on lower reversibility of adsorption for phenol and 2-CP compared with 2-NP in the case of SA4. The absence of oxidative coupling resulted in a higher reversibility of 2-NP adsorption. The oxidative coupling reactions were obviously not effective in the case of chemically activated carbon CA1. In Section 4.1.3.6, this was attributed to the affinity of thermally activated carbons towards oxygen (Jonge et al., 1996b) since thermal activation is carried out in the absence of oxygen and leads to a more reactive surface. However, chemically activated carbons have already a surface with fully oxidized active sites so that interaction with oxygen does not affect the surface.

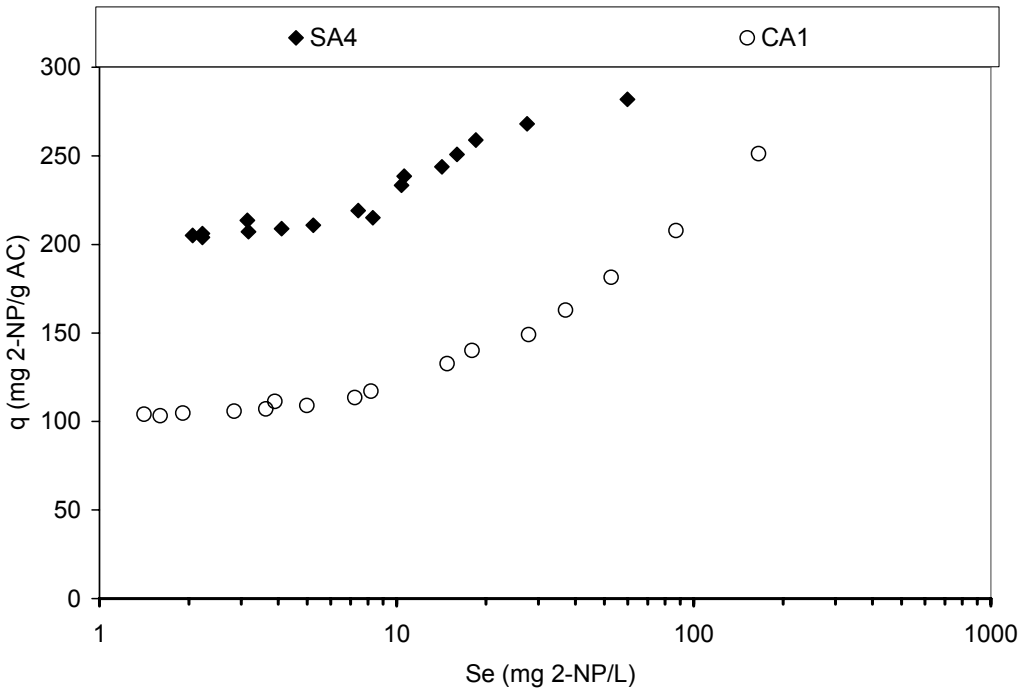


Figure 4.42. 2-Nitrophenol desorption isotherms for activated carbon types SA4 and CA1.

Table 4.18. Freundlich isotherm constants for 2-nitrophenol desorption.

Carbon Type	Activation method	K [(mg/g) (L/mg)$^{1/n}$]	1/n	R^2	Desorption Efficiency (%)
SA4	Thermal	184.98	0.1033	0.92	34.59
CA1	Chemical	87.12	0.1811	0.93	69.07

4.4.3. Biodegradation and Bioregeneration Studies with 2-Nitrophenol

All biodegradation and bioregeneration runs with 2-NP were performed in the presence of phenol (see Table 3.6 in Section 3.6.3), except in the biodegradation reactor in RUN 18, where 2-NP was used as the only carbon source. Phenol was also used in these studies, because 2-NP was not biodegraded with non-acclimated sludge when it was used as the only carbon source and an acclimation procedure was required in the presence of phenol. This acclimation procedure was explained in Section 3.6.3.

4.4.3.1. Biodegradation of 2-Nitrophenol. RUN 16 was performed to investigate the effect of acclimation on bi-solute biodegradation of phenol and 2-NP. As explained in Section 3.6.3, the acclimation procedure resulted in a change in microbial population. Apparently, phenol-oxidizers dominated the biomass population, as in the case of acclimation for 2-CP. This acclimated activated sludge (Acc. AS) provided biodegradation of 2-NP, which was not possible with the non-acclimated activated sludge (Non-Acc. AS), although non-acclimated biomass could biodegrade phenol. Figure 4.43a shows that biodegradation of both phenol and 2-NP were much better with acclimated biomass. Most of the phenol was removed in 24 hours and all of the phenol was removed at the end of 48 hours in bidegradation reactors with acclimated biomass (Figures 4.43a and 4.44a). However, there was a lag phase of about 24-48 hours for biodegradation of phenol with the non-acclimated sludge (Figure 4.43a). This lag phase was due to inhibition by 2-NP, because it was not observed when phenol was the only carbon source in Section 4.1.3.1. But phenol was completely removed by the acclimated biomass after this lag period.

On the other hand, 2-NP was not removed even by the acclimated biomass during the first 24 hours, but was removed completely at the end of 6 days of aeration after depletion of phenol (Figures 4.43a and 4.44a). Phenol-oxidizers apparently preferred phenol as the carbon source instead of 2-NP. This finding was similar to the case of bi-solute biodegradation of 2-CP and phenol in Section 4.3.4.1. However, 2-NP removal was very low in the case of non-acclimated biomass (Figure 4.43a). The effect of acclimation can also be seen in Figure 4.43b, which shows much better COD removals together with higher oxygen uptake rates during the first hours of aeration in the reactor receiving acclimated biomass.

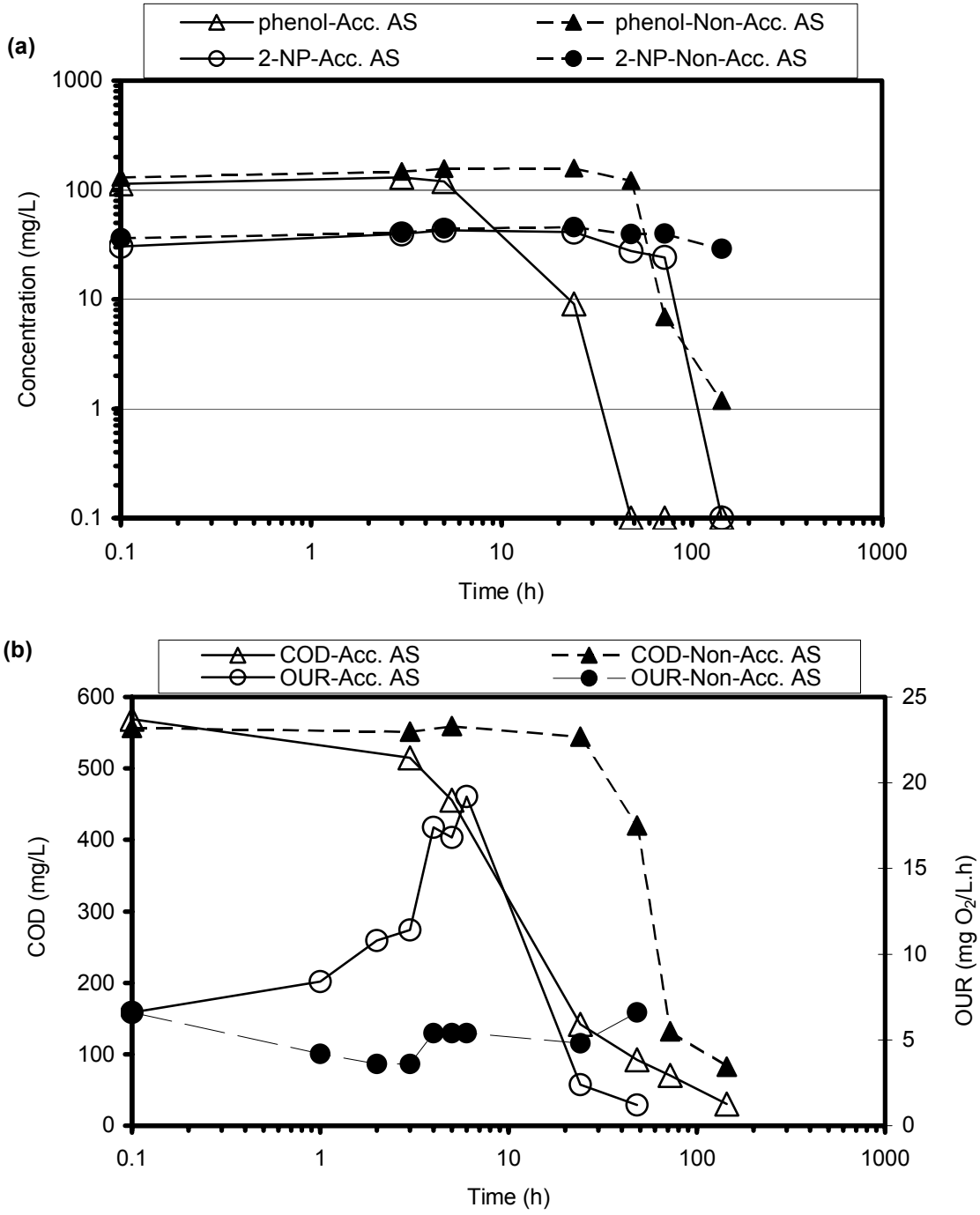


Figure 4.43. a) Phenol and 2-NP profiles b) COD and OUR profiles in biodegradation reactors receiving acclimated (Acc. AS) and non-acclimated (Non-Acc. AS) activated sludges in RUN 16.

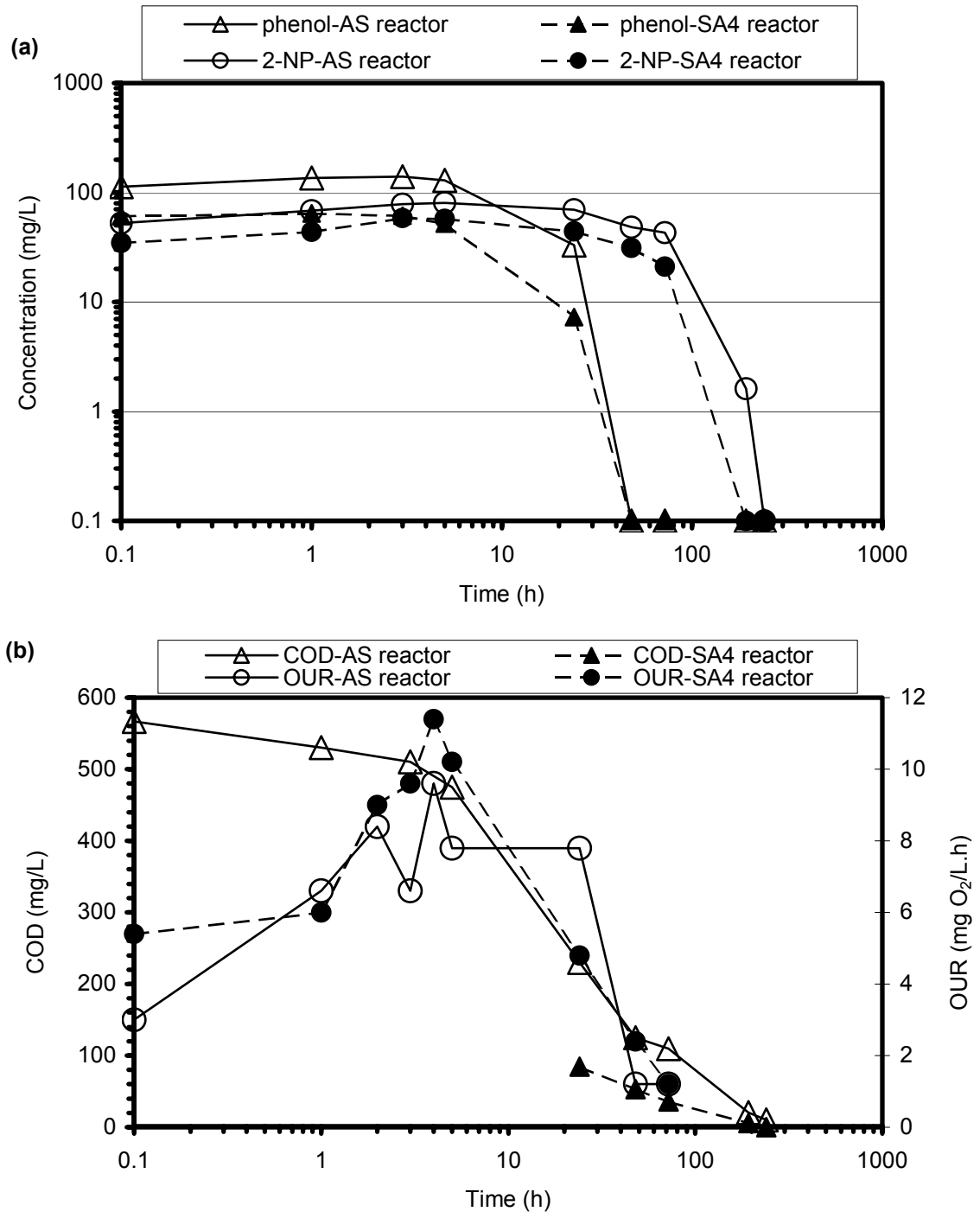


Figure 4.44. a) Phenol and 2-NP profiles b) COD and OUR profiles in biodegradation reactor and bioregeneration reactor with 2000 mg/L SA4 (powdered, thermally activated) in RUN 17.

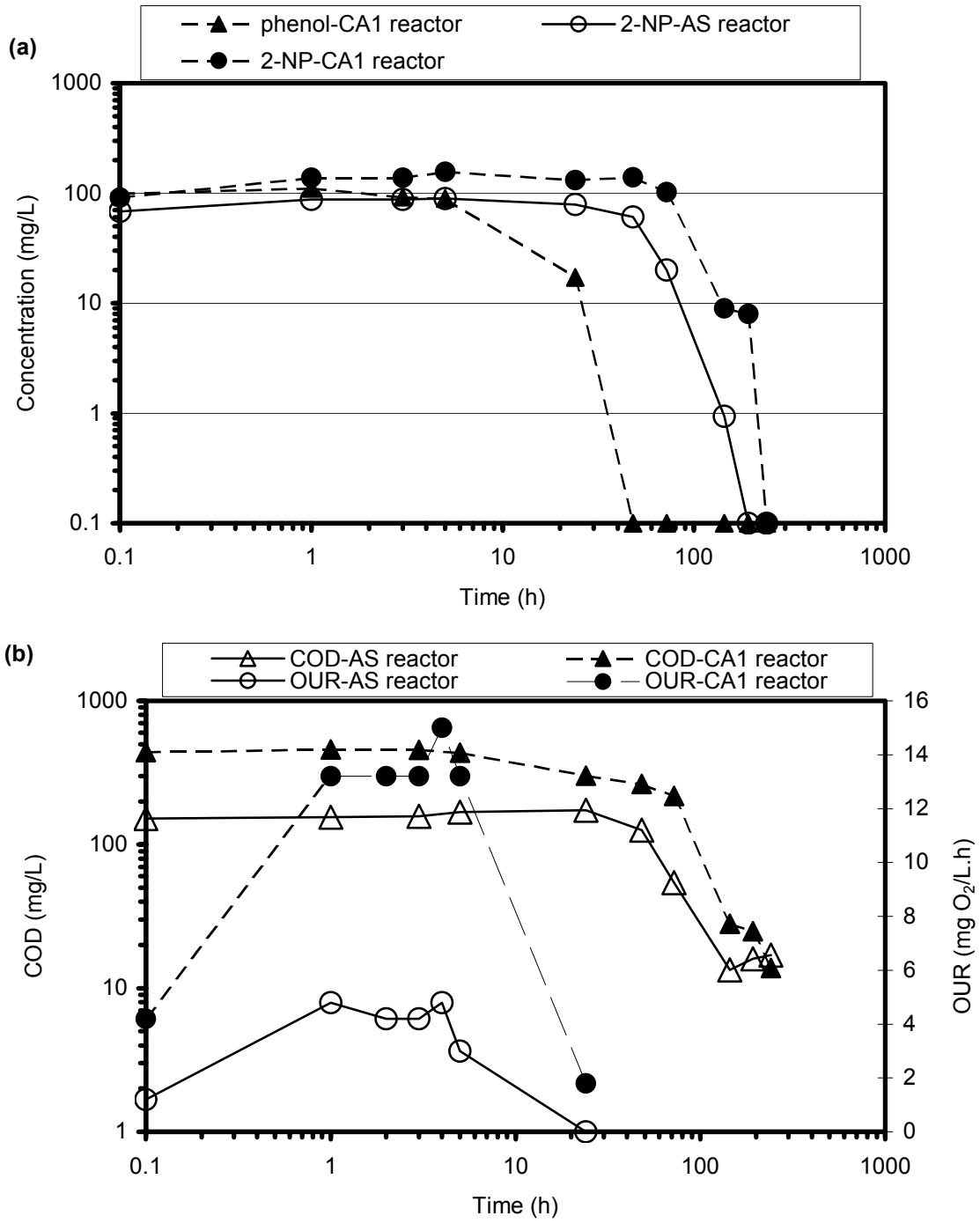


Figure 4.45. a) Phenol and 2-NP profiles b) COD and OUR profiles in biodegradation reactor (receives 2-NP as the only carbon source) and bioregeneration reactor with 2000 mg/L CA1 (powdered, chemically activated) in RUN 18

The biodegradation reactor in RUN 18 received about 100 mg/L 2-NP as the only carbon source in order to investigate the occurrence of cometabolism in biodegradation of 2-NP. 2-NP concentration decreased very slowly during the first 24 hours of aeration, but decreased particularly after 24-48 hours of aeration down to below 1 mg/L at the end of 6 days of aeration (Figure 4.45a). This single-solute removal pattern of 2-NP was very similar to the bi-solute removal patterns in Figures 4.43a and 4.44a. Cometabolic metabolism seemed to be valid in the case of 2-NP removal, as in the case of 2-CP removal in Section 4.3.4. Phenol-oxidizers of the acclimated biomass could remove 2-NP even in the absence of phenol with a lag phase as also evidenced in the presence of phenol. Non-specific phenol monooxygenase enzymes, which were produced during the acclimation phase, acted in the biodegradation of 2-NP according to the resting cell condition.

The zero-order biodegradation rate constants for phenol removal were determined particularly for the first 24 hours of aeration when substrate concentration was sufficiently high. These were calculated to be 5.81 and 5.05 mg phenol/L.h, in biodegradation reactors receiving acclimated sludge in RUNS 16 and 17, respectively. However, the zero-order biodegradation rate constant for the non-acclimated sludge in RUN 16 was only 1.5 mg phenol/L.h between 24-48 hours of aeration. Zero-order biodegradation rates were also calculated for 2-NP after 24 hours of aeration when biodegradation of 2-NP started. These were calculated to be about 0.33 and 0.40 mg 2-NP/L.h, in biodegradation reactors receiving acclimated sludge in RUNS 16 and 17, respectively. The 2-NP biodegradation rate constant for the non-acclimated sludge in RUN 16 was only 0.13 mg 2-NP/L.h. In the absence of phenol, the 2-NP biodegradation rate was 0.67 mg 2-NP/L.h, which was even higher compared with bi-solute biodegradation. 2-NP biodegradation rates were much lower than phenol biodegradation rates as expected. 2-NP was removed more slowly, because it could be removed only after depletion of phenol.

COD removal also followed zero-order biodegradation kinetics during the first 24 hours of aeration in parallel to high biodegradation of phenol with acclimated sludge during this period. The zero-order biodegradation rate constants during the first 24 hours of aeration were calculated to be 17.59 and 13.70 mg COD/L.h in RUNS 16 and 17, respectively, where the S_0/X_0 ratios were 0.59 mg COD/mg MLSS. Almost no COD removal was seen with the non-acclimated sludge in RUN 16 (Figure 4.43a) during this

period of lag phase, and removal rate was only 8.59 mg COD/L.h following the lag phase. COD removal rate was only 3.98 mg COD/L.h when 2-NP was the only carbon source in the biodegradation reactor in RUN 18. COD biodegradation rate was much slower in that case because 2-NP removal was very slow compared with phenol removal. At the end of aeration, about 10-30 mg/L and 36 mg/L non-phenolic COD remained in the biodegradation reactors with acclimated and non-acclimated sludge, respectively. These stemmed from side-products of phenol and 2-NP removal or soluble microbial products (SMP).

4.4.3.2. Removal of Phenol and 2-Nitrophenol in Bioregeneration Reactors. Phenol and 2-NP removals in bioregeneration reactors (Figures 4.44a-4.46a) had a similar trend as in biodegradation reactors; such that phenol was removed very rapidly during the first 24-48 hours and 2-NP removal started after 24 hours. Particularly, in RUN 18 with CA1, 2-NP removal started after 48 hours (Figure 4.45a), because bulk 2-NP concentration was higher during this period (above 100 mg/L) compared with other bioregeneration runs. The results of RUN 19 show a comparison between bioregeneration of thermally (SA4) and chemically activated (CA1) carbons (Figure 4.46). Phenol and 2-NP concentrations in the bulk liquid were always much lower in bioregeneration reactor containing SA4 in relation to the much lower desorption efficiency for this type of carbon (Tables 4.4 and 4.18).

Bulk 2-NP concentrations during the first 24 hours of aeration in the bioregeneration reactors of RUN 19 (3-6 mg/L in SA4 reactor, 20-35 mg/L in CA1 reactor) were much lower compared with those in RUN 17 (45-60 mg/L in SA4 reactor) and RUN 18 (130-155 mg/L in CA1 reactor). The reason for this was that more 2-NP (1000 mg) was used in the initial adsorption of 4 grams of activated carbon before beginning the bioregeneration RUNS 17 and 18 compared with RUN 19 (400 mg 2-NP was used in pre-adsorption of 4 g of each activated carbon type). In RUN 19, less 2-NP was adsorbed (see Table 3.6) before bioregeneration, but obviously they were adsorbed more irreversibly with greater adsorption energies compared with RUNs 17 and 18. Hence, less 2-NP was desorbed during bioregeneration in this run. This finding showed the importance of pre-loading on bioregeneration of activated carbon. Non-phenolic COD, which stemmed from microbial products, was between 5-13 mg/L in bioregeneration reactors. This was lower compared

with the biodegradation reactors (10-30 mg/L), because SMP were probably adsorbed on the bioregenerated sites of activated carbon.

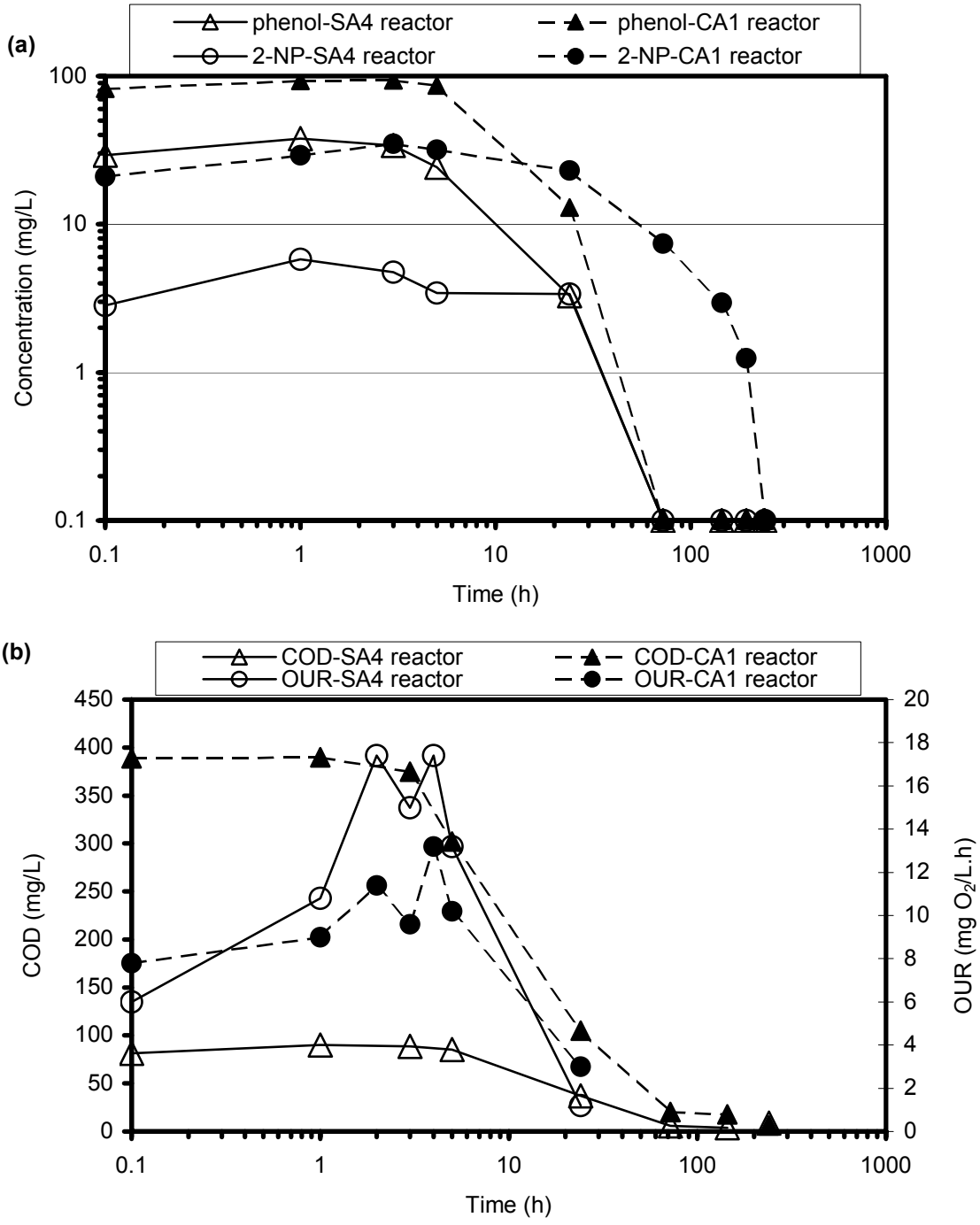


Figure 4.46. a) Phenol and 2-NP profiles b) COD and OUR profiles in bioregeneration reactors with SA4 (powdered, thermally activated) and CA1 (powdered, chemically activated) in RUN 19.

Although, phenol and 2-NP concentrations were higher in the biodegradation reactor of RUN 17 (Figure 4.44a), oxygen uptake rates were higher in the parallel bioregeneration reactor with SA4 (Figure 4.44b) during the first hours of aeration. Also in RUN 19, higher bulk concentrations in the reactor with CA1 (Figure 4.46a) resulted in lower OURs compared with the reactor with SA4 (Figure 4.46b) during the same initial period. Considering that phenol did not cause inhibition of acclimated sludge, it can be deduced that higher 2-NP concentrations caused this inhibition. High OURs during the first hours of aeration were mostly caused by phenol biodegradation because OURs were very low (below 5 mg/L.h) in the biodegradation reactor receiving only 2-NP in RUN 18 (Figure 4.45b). 2-NP biodegradation was very slow so that it did not result in notable OURs as in the case of 2-CP biodegradation. However, lower desorbability of 2-CP seemed to result in less inhibition in Section 4.3.4 compared with 2-NP.

4.4.3.3. Quantification of Bioregeneration. Direct measurement of bioregeneration was possible through the use of post-bioregeneration adsorption experiments (Section 3.6.3). These experiments enabled comparison of adsorption capacities before and after bioregeneration (Eq. 2.7 in Section 2.3.6.2). The direct measurement method resulted in bioregeneration efficiencies at the end of 10 days of aeration as tabulated in Table 4.19. Initial conditions in these bioregeneration runs can be seen in Table 3.6. Bioregeneration efficiencies were higher considering the total of phenol and 2-NP loadings in the case of chemically activated CA1 compared with thermally activated SA4 (Table 4.19), as in the previous studies with phenol and 2-CP. This was also in accordance with higher desorbabilities (Table 4.4 and 4.18) of two competing compounds from the chemically activated carbons. Considering both phenol and 2-NP, bioregeneration efficiencies were higher than the desorbabilities of phenol and 2-NP, particularly in the case of thermally activated carbon SA4. Phenol and 2-NP desorbabilities from SA4 were only about 20 % (Table 4.4) and 35 % (Table 4.18), respectively. This finding is also in agreement with the previous results with phenol and bi-solute bioregeneration of phenol and 2-CP. The increase in desorbabilities in the presence of microorganisms, which resulted in higher bioregeneration capacities, can only be attributed to the exoenzymatic bioregeneration of thermally activated carbons. The exoenzymatic bioregeneration of chemically activated carbons seemed to be less plausible with 2-NP as with phenol and 2-CP.

Table 4.19. Bioregeneration rates and efficiencies for activated carbons competitively loaded with phenol and 2-nitrophenol.

Run No	Carbon type	Bioregen. Efficiency for Phenol loading (%)	Bioregen. Efficiency for 2-NP loading (%)	Bioregen. rate constant for Phenol loading, k (h^{-1})	Bioregen. rate constant for 2-NP loading, k (h^{-1})
17	SA4	19.5	64.9	0.0173	0.0021
18	CA1	90.0	64.7	0.0376	0.0083
19	SA4	32.6	84.9	0.0184	n.a.
19	CA1	82.3	89.8	0.0409	0.0086

n.a.: Rate constants were not applicable in some cases because of low correlation (R^2<0.75).

Bioregeneration efficiencies were calculated to be higher for the 2-NP loadings compared with the phenol loadings in the case of thermally activated carbon SA4 (Table 4.19). This was also in accordance with higher 2-NP desorbabilities from these carbons (Table 4.18). Compared with phenol and 2-CP, higher 2-NP desorbabilities was previously attributed to the absence of oxidative coupling reactions in the case of 2-NP (Section 4.4.2). The bioregeneration experiments showed that oxidative coupling reactions were also very effective on the extent of bioregeneration. The absence of these reactions on the activated carbon surface make 2-NP an ideal compound in terms of bioregenerability.

The first-order bioregeneration rate constants were calculated both for phenol and 2-NP with high correlations (R^2= 0.95-1) as explained in Section 4.1.3.3. Bioregeneration rate constants for phenol were much higher than 2-NP (Table 4.19). This was due to higher biodegradation rate of phenol. Bioregeneration rate constants for phenol in the presence of 2-NP (Table 4.19) were about the same order of magnitude as in the single-solute case (Table 4.5), but were much lower than the bi-solute case in the presence of 2-CP (Table 4.15). This showed that in bi-solute bioregeneration of phenol and 2-CP, 2-CP was adsorbed with a much higher adsorption energy during the initial adsorption step (Section 4.3.4) when compared with 2-NP adsorption in the presence of phenol. In bi-solute case of phenol and 2-CP, almost all of the phenol had been desorbed (Table 4.14) due to much lower adsorption energies compared with 2-CP. However in bi-solute case with phenol and 2-NP, it can be suggested that adsorption energies of two substances were comparable. Therefore, phenol bioregeneration rates were not higher in the competitive presence of 2-NP compared with the single-solute case.

For comparison purposes, first-order biodegradation rate constants were also calculated both for phenol and 2-NP considering the total aeration period during bi-solute biodegradation in RUNS 16-18. Phenol biodegradation rate constants were found as 0.1304 h^{-1} and 0.0695 h^{-1} (R^2=1) and 2-NP biodegradation rate constants were found as 0.0095 h^{-1} and 0.0216 h^{-1} (R^2=0.93-0.96) with acclimated sludge in RUNs 16 and 17, respectively. These first-order biodegradation rate constants were higher than the bioregeneration rate constants in Table 4.19. Hence, desorption was the rate-limiting step in bi-solute bioregeneration of phenol and 2-NP as in the case of single-solute bioregeneration of phenol (Section 4.1.3.5). But that was in contrast to the bi-solute bioregeneration of phenol and 2-CP (Section 4.3.4.4). This finding can be also attributed to comparable adsorption energies for phenol and 2-NP, which resulted in an adsorption competition between phenol and 2-NP. Hence desorption became rate-limiting in bioregeneration. This was not the case in bi-solute bioregeneration of phenol and 2-CP, because 2-CP was adsorbed preferentially with much higher adsorption energies compared with phenol as discussed previously in Section 4.3.

Also the first-order COD biodegradation rate constants were calculated as 0.0204 h^{-1} (R^2=0.87) and 0.0157 h^{-1} (R^2=0.97) in RUNs 16 and 17, respectively, considering the total aeration period of 10 days. These were closer to 2-NP biodegradation rates because phenol biodegradation ceased at the end of 24-48 hours, and hence a great portion of COD consisted of 2-NP after depletion of phenol. First-order biodegradation rates with the non-acclimated sludge in RUN 16 were 0.0429 h^{-1} (R^2=0.89) for phenol, 0.0036 h^{-1} (R^2=0.96) for 2-NP and 0.0146 h^{-1} (R^2=0.92) for COD. These were lower than the rate constants for acclimated sludge. First-order biodegradation rates when 2-NP was the only carbon source in RUN 18 were 0.0102 h^{-1} (R^2=0.88) for 2-NP and 0.0141 h^{-1} (R^2=0.89) for COD.

At the end of bioregeneration runs, activated carbon samples were taken for solvent extraction (Section 3.6.3) in order to determine the extractable phenol and 2-NP loadings remaining on activated carbons. Extracted phenol and 2-NP loadings were proportioned to the initial loadings (Q_1) in bioregeneration runs. No phenol was extracted from the CA1 carbon samples in RUNS 18 and 19. On the other hand, only 0.74 % of initial 2-NP loading was extracted from CA1 reactor in RUN 19 and no 2-NP was extracted from CA1

in RUN 18. In case of thermally activated SA4, only 0.74 % of initial phenol and 2 % of initial 2-NP was extracted by the solvent. Most of the extractable phenol and 2-NP was removed during bioregeneration, and further solvent extraction did not improve their desorbability. The non-extractable phenol and 2-NP remaining on carbons even after extraction should be considered as chemically adsorbed molecules. Solvent extraction had resulted in higher efficiencies for 2-CP in section 4.3.4.4. This also indicated higher adsorption energies for 2-CP compared with both phenol and 2-NP such that bioregeneration process could not remove some of the 2-CP (less than 10%), which solvent extraction could remove.

5. CONCLUSIONS AND RECOMMENDATIONS

For phenolic compounds used in this study, thermally activated carbons were better adsorbers than chemically activated ones. This was attributed to the presence of acidic surface functional groups on chemically activated carbons. However, chemically activated carbons possessed a very high desorbability compared with thermally activated carbons. Reversibility of adsorption was dependent on the carbon activation method and chemical surface characteristics of carbon rather than the physical form. Chemisorption was the dominant adsorption mechanism for thermally activated carbons, whereas physisorption was dominant for chemically activated carbons.

Adsorption and desorption capacities did not differ much between the powdered and granular countertypes. Comparison of powdered carbons with their granular countertypes resulted in slightly higher degrees of hysteresis for the powdered activated carbons, either thermally or chemically activated. This showed only slightly less desorbability from the PAC compared to a similar GAC with a more macroporous structure. On the other hand, both adsorption and desorption were faster for the PACs, probably due to higher intraparticle diffusivity compared to GACs with higher diameters. However, the diffusivity controlled only the adsorption and desorption rates, but not the total adsorbability and desorbability. On the other hand, reversibility of adsorption was found to increase with the carbon dose for the experimental conditions of the study. This was attributed to the high adsorption energies at lower carbon doses with lower phenol loadings.

The findings of the study revealed that bioregeneration of activated carbon was greatly dependent on carbon activation type. Chemically activated carbons were more bioregenerable than thermally activated ones in relation to higher desorbabilities from these carbons. A novelty of this study was the comparison of PAC and GAC countertypes with similar physical characteristics in terms of bioregeneration, which was not previously investigated in literature. The results revealed comparable bioregenerabilities for PAC and GAC countertypes. This showed that carbon size was not an effective factor for bioregenerability.

Bioregeneration was controlled by the reversibility of adsorption. It was certain that desorption served as a prerequisite step for bioregeneration and desorption proceeded due to a concentration gradient. Since biological activity is responsible for the concentration gradient created between the carbon's surface and bulk liquid, this desorption-biodegradation sequence should certainly be regarded as bioregeneration. However, bioregeneration of thermally activated carbons was much higher than their total desorbabilities. This indicated that some exoenzymatic reactions had occurred so that bioregeneration exceeded the expectation. Considering that phenolic compounds used in this study are adsorbed mainly on micro- and mesopores and exoenzymes are not expected to reach micropores, exoenzymatic reactions are expected to take place in the mesopores. In the case of chemically activated carbons, exoenzymes were not effective on bioregeneration since adsorption was already highly reversible and the non-desorbed phenol was probably existing in the micropores where enzymes could not reach. As a novelty, the results of this study indicated that exoenzymatic bioregeneration was very much related to the carbon activation type, adsorption characteristics and porosity of carbon. Exoenzymatic bioregeneration is still a hypothesis because enzyme reactions inside the pores cannot be measured directly.

Removal of 2-chlorophenol by adsorption was easier than phenol. However, its desorption, and consequently its bioregeneration was more difficult. Biodegradation and bioregeneration studies showed that biodegradation of 2-chlorophenol, as the only carbon source, was very difficult and very slow. Hence, bioregeneration of activated carbon did not occur with a non-acclimated biomass under the conditions of this study when 2-chlorophenol was the sole carbon source. But the same biomass could successfully bioregenerate activated carbons loaded with phenol. Bioregeneration was greatly dependent on the biodegradability of the target phenolic compound.

A significant result of this study is that the dependency of adsorbability and desorbability on carbon activation type can play a crucial role in treatment systems receiving phenolic compounds. In treatment of aromatic compounds by adsorption processes that are not combined with biological processes, thermally activated carbons should be preferred. However, in biological processes combined with activated carbon adsorption processes, chemically activated carbons should be preferred to increase the

service life of activated carbon. But, it should be noted that this statement is true only if the target aromatic compounds can be biologically degraded in these systems, as in the case of phenol. Since 2-chlorophenol was not sufficiently biodegraded in the absence of a metabolic substrate, bioregeneration of activated carbon loaded totally with 2-chlorophenol will not be a realistic application. It can be deduced that the type of the target compound was very important on the extent or occurrence of bioregeneration. Therefore, for the treatment of 2-chlorophenol alone, thermally activated carbons should be preferred without the need of a combination with biological processes. But, on the other hand, chemically activated carbons combined with biological processes may suggest a more efficient treatment of phenol.

Bi-solute adsorption studies investigated the competitive adsorption of two phenolic compounds; phenol and 2-chlorophenol. The bi-solute adsorption of 2-chlorophenol was not negatively affected by the presence of phenol, although phenol adsorption was suppressed to a great extent. This showed that 2-chlorophenol was adsorbed preferentially by each type of activated carbon, but phenol was adsorbed competitively. The SIAS (Simplified Ideal Adsorbed Solution) model underestimated the 2-chlorophenol loadings on each type of activated carbon and overestimated phenol loadings, particularly those on the thermally activated carbons SA4 and PKDA. Competition between phenol and 2-chlorophenol was non-ideal and in favor of 2-chlorophenol. Irreversible adsorption and unequal competition for adsorption sites were the main reasons for the failure of the SIAS model. On the other hand, the ISIAS (Improved Simplified Ideal Adsorbed Solution) model accounting for non-ideal competition for adsorption sites provided a reasonable fit for bi-solute data. A modified ISIAS model was also used considering solubilities of competing phenol and 2-chlorophenol. But, this model was not successful in describing the bi-solute data for the chemically activated carbons, although it could reasonably predict data for thermally activated ones. The conditional applicability of several models was not tested before in literature in the case of carbons subjected to different types of activation. In the present study, the type of the carbon was found to be very important and decisive for the choice of either the improved and modified forms of the ideal adsorbed solution model and in the application of competition factors.

On the other hand, more phenol was desorbed in the bi-solute case, but desorption of 2-chlorophenol was less compared with single-solute desorption for each type of activated carbon. Therefore, it was suggested that 2-chlorophenol was preferentially adsorbed on adsorption sites with greater energy, and phenol was adsorbed on less energetic sites so that phenol was more readily desorbed. Combination of adsorption and desorption studies showed that both phenol and 2-chlorophenol were mainly adsorbed on micro and mesopores rather than macropores. A novelty in this study was investigating the effect of activated carbon type on competitive adsorption and desorption of a bi-solute mixture and also on the use of models to predict competitive adsorption.

Oxidative coupling reactions seemed to lead to the lower reversibility of adsorption for phenol and 2-chlorophenol in the case of thermally activated carbons. However, 2-nitrophenol was not subject to oxidative coupling reactions. The absence of oxidative coupling resulted in a higher reversibility of 2-nitrophenol adsorption compared with 2-chlorophenol and phenol. However, this was not observed in the case of chemically activated carbons. The oxidative coupling reactions were obviously not effective in the case of chemically activated carbons. This study showed that the occurrence of irreversible adsorption due to oxidative coupling of phenolic substances was also related to the type of carbon activation. Another novelty of this study was investigating the effect of oxidative coupling reactions on the extent of bioregeneration. The absence of oxidative coupling reactions also resulted in high bioregeneration of thermally activated carbons loaded with 2-nitrophenol compared with phenol and 2-chlorophenol.

In competitive biodegradation, microorganisms apparently preferred the more biodegradable phenol instead of 2-chlorophenol and 2-nitrophenol. 2-chlorophenol and 2-nitrophenol were only removed by acclimated microorganisms and by a cometabolic pathway in the presence of phenol. Acclimation resulted in domination of phenol-oxidizing bacteria. Phenol was a good growth substrate for cometabolism of chlorophenols and nitrophenols because of structural analogy. Therefore, bioregeneration of activated carbon loaded with 2-chlorophenol and 2-nitrophenol could be achieved only in the presence of acclimated microorganisms and phenol as the growth substrate. An original and important finding of this study was investigating the effect of the presence of a metabolic substrate

and cometabolic biodegradation of non-growth phenolic compounds during bioregeneration of activated carbon.

When phenol was the only substrate, desorption was a limiting factor for bioregeneration rate. However, in the bi-solute case of phenol and 2-chlorophenol, biodegradation was the rate-limiting factor for bioregeneration. This was because phenol was adsorbed on less energetic adsorption sites compared with 2-chlorophenol, and hence desorption of phenol was easier in the bi-solute case. On the other hand, biodegradation of 2-chlorophenol was very slow, so that biodegradation was rate-limiting for bioregeneration rather than desorption. However, desorption was the rate-limiting step in bi-solute bioregeneration of phenol and 2-nitrophenol as in the case of single-solute bioregeneration of phenol. This was attributed to comparable adsorption energies for phenol and 2-nitrophenol, which resulted in an adsorption competition between phenol and 2-nitrophenol. Hence, desorption became rate-limiting in bioregeneration. Also, concentration of the phenolic compounds in the initial loading of activated carbon determined the adsorption energies of competing phenolic species. The results showed that adsorption energies and initial loading conditions were very effective on the extent of bioregeneration. Both desorption and biodegradation may be rate-limiting in bioregeneration depending on initial adsorption conditions and biodegradability of compounds.

Solvent extractions showed that there was a considerable amount of chemically adsorbed molecules, which could not be desorbed and hence could not be bioregenerated. Considering the lower bioregeneration and desorption efficiencies for thermally activated carbons, these solvent extraction studies showed that chemical adsorption was more plausible on thermally activated carbons compared with chemically activated ones.

This study is an example of an offline bioregeneration where activated carbon is first loaded with a substance and then subjected to bioregeneration. However, in conventional biological activated carbon filters and PAC added activated sludge systems, biodegradation, adsorption and desorption occur simultaneously. Conventional systems, which consume substantial amounts of activated carbon, can be combined with additional offline bioregeneration facilities to decrease the costs related to activated carbon. The

results of this study showed that bioregeneration of activated carbon can be successfully achieved in the treatment of phenolic wastewaters. In wastewater treatment plants, which employ activated carbon adsorption for the removal of phenolic substances, bioregenerability of these compounds may provide a cost-effective operational flexibility. These results can possibly be extended to offline carbon bioregeneration in the treatment of drinking waters contaminated with phenolic substances.

In future work, it may be recommended to investigate the bioregeneration in biologically activated carbon (BAC) filters loaded with the phenolic compounds used in this study. The results obtained in this study may provide a basis for a pilot-scale study, which would investigate the increase in service life of a GAC column due to bioregeneration. Also, other aromatic compounds or aliphatic compounds can be studied in future works in order to extend the investigation of the bioregenerability of activated carbon loaded with various compounds. Particularly, bioregeneration due to exoenzymes is a very interesting issue, which may require further studies. Future research is still required to verify the validity of exoenzymatic reactions inside carbon pores. It is recommended to use molecular tools or protein measurements in future works in order to determine the presence of enzymes inside or on carbon surfaces. Engineered use of enzymes for the purpose of bioregeneration of activated carbon might be a challenging innovation in activated carbon applications.

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APPENDIX A: CALIBRATION CURVES AND CHROMATOGRAMS

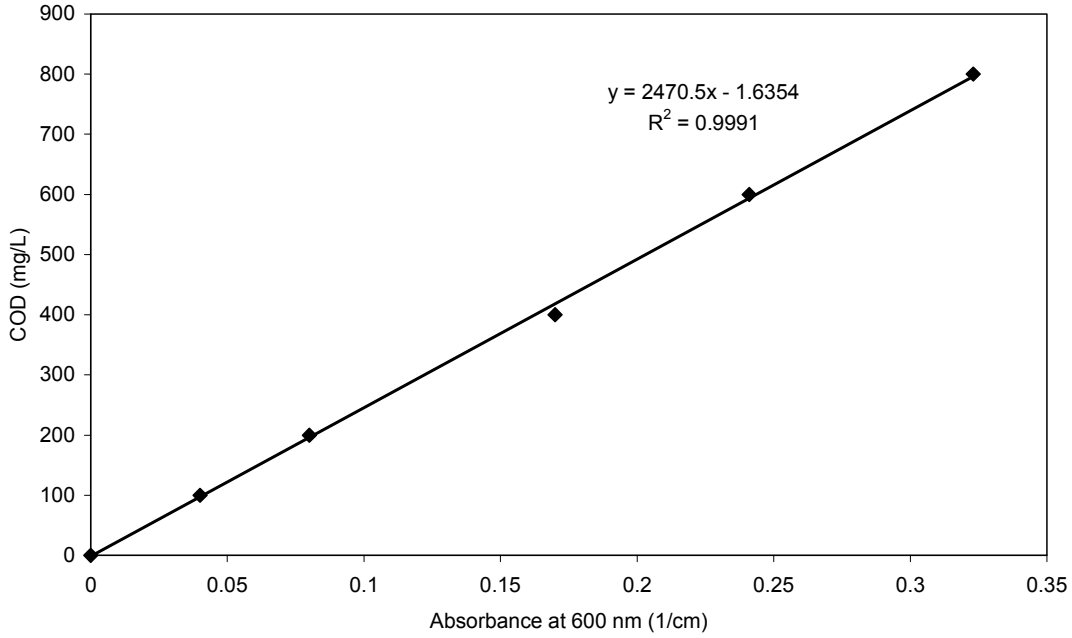


Figure A.1. Calibration curve for high range chemical oxygen demand (COD) analysis.

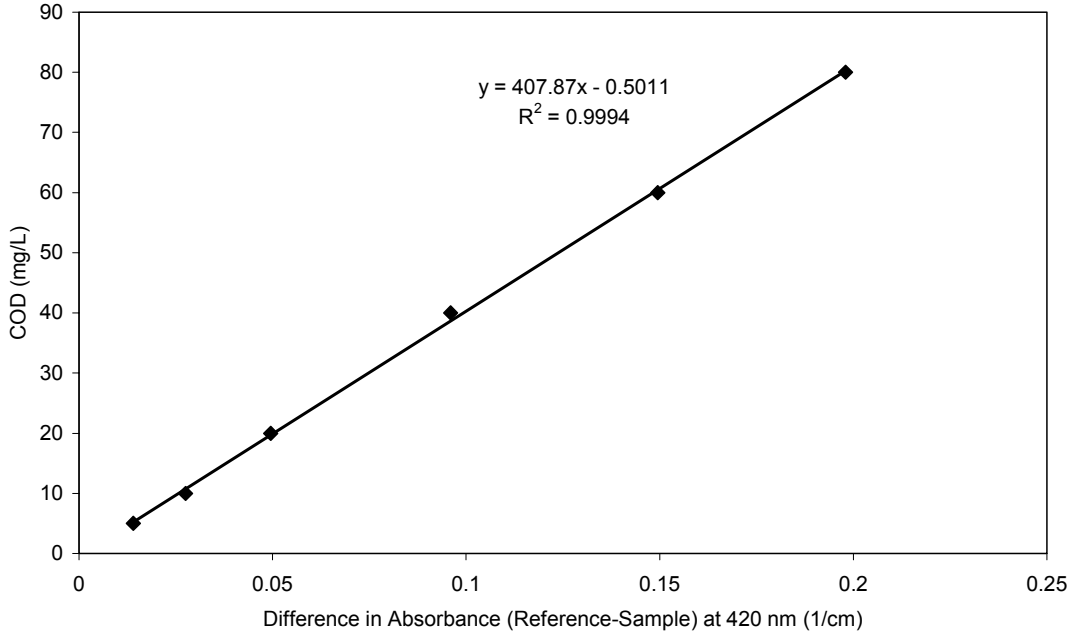


Figure A.2. Calibration curve for low range chemical oxygen demand (COD) analysis.

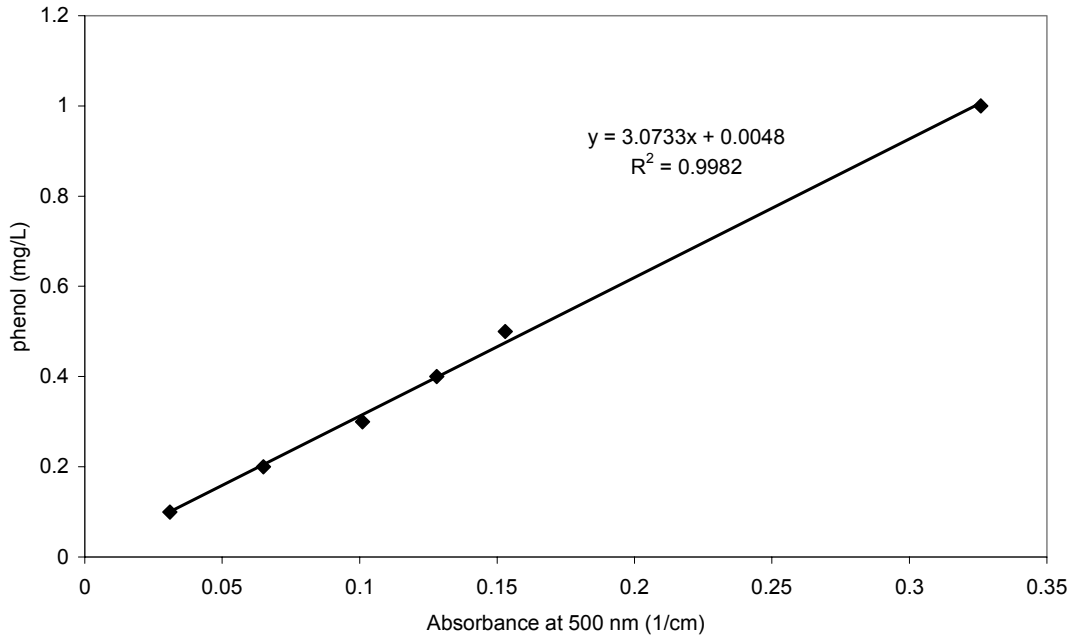


Figure A.3. Calibration curve for phenol analysis with the 4-aminoantipyrine method within the concentration range of 0.1-1 mg/L.

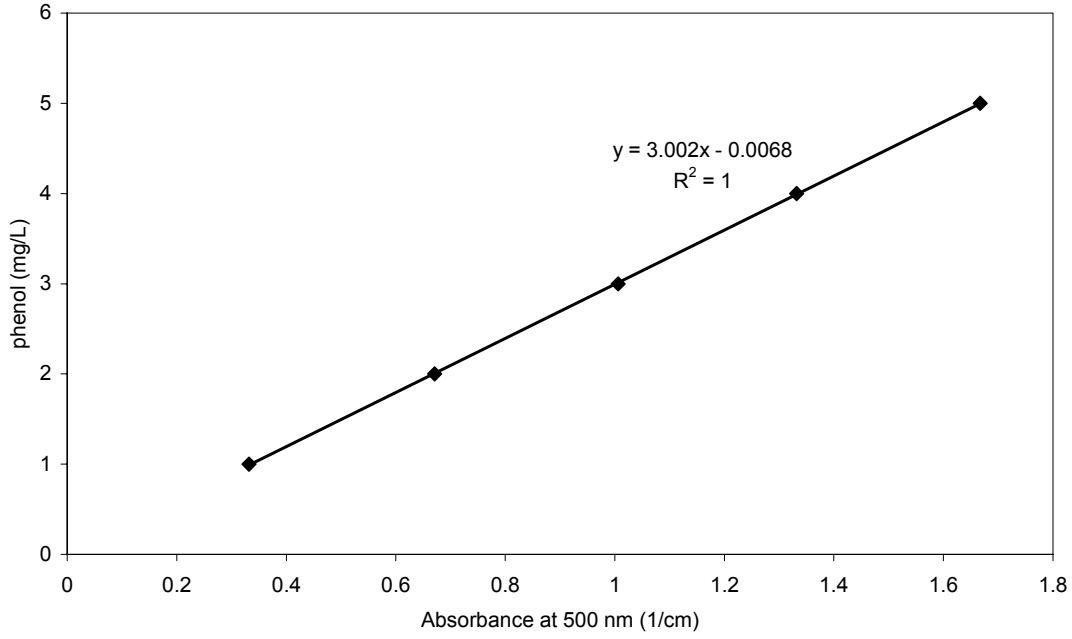


Figure A.4. Calibration curve for phenol analysis with the 4-aminoantipyrine method within the concentration range of 1-5 mg/L.

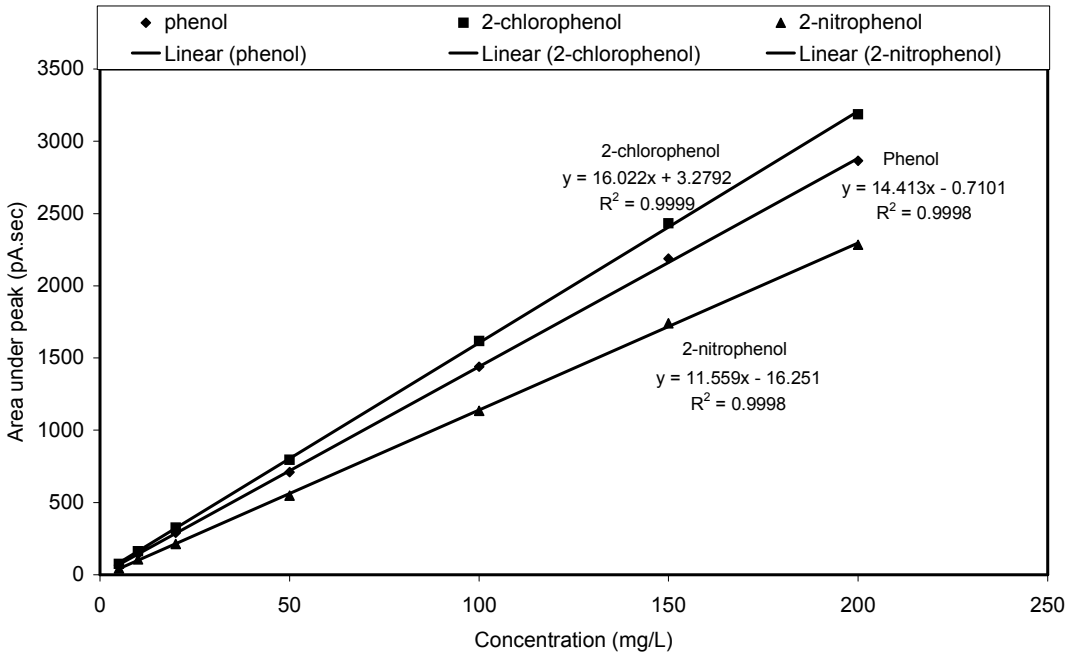


Figure A.5. Calibration curves for gas chromatographic analysis.

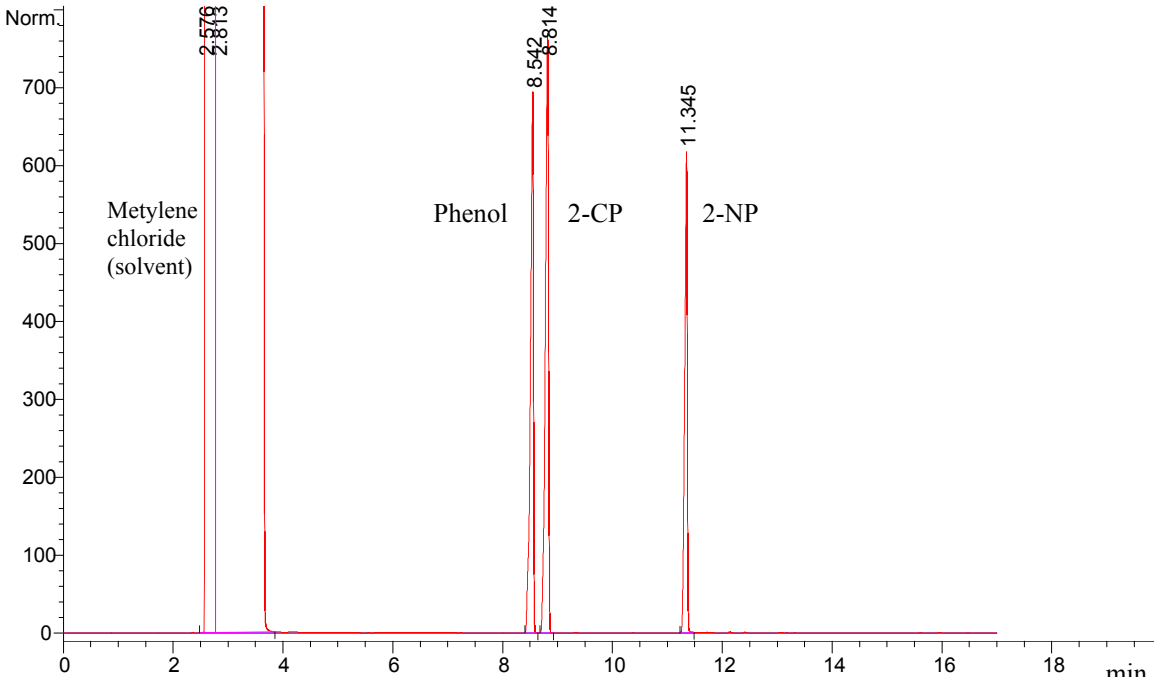


Figure A.6. Example of a gas chromatogram for a sample containing 200 mg/L of each compound; phenol, 2-chlorophenol (2-CP) and 2-nitrophenol (2-NP).

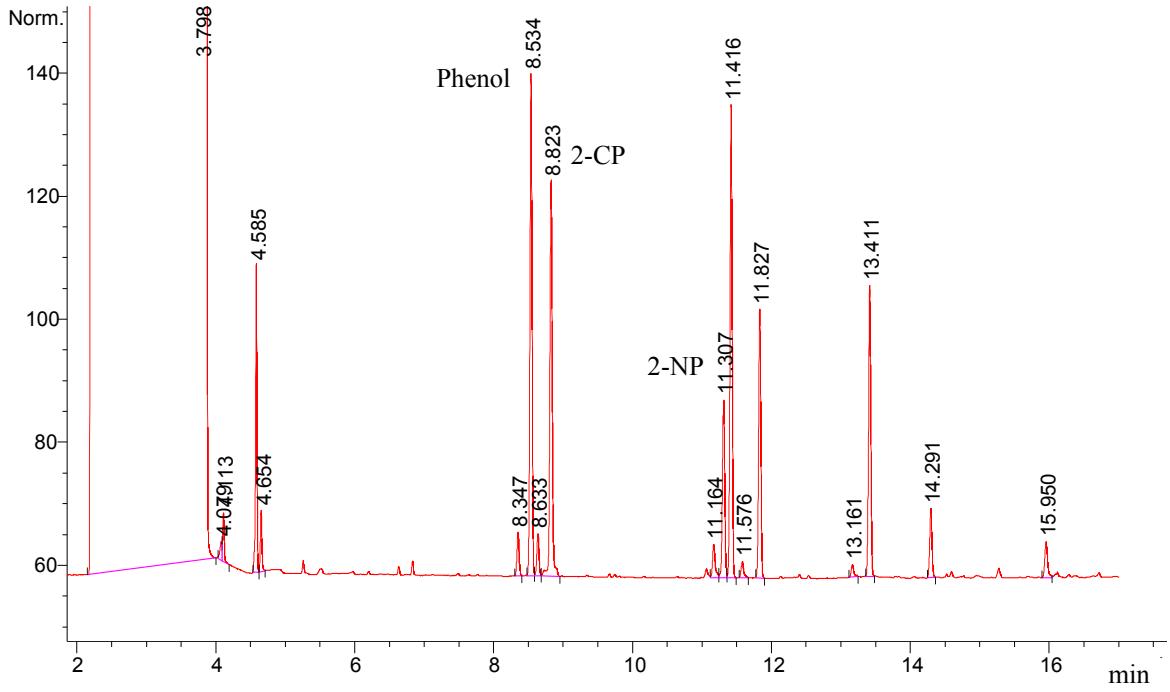


Figure A.7. Gas chromatogram of mixed phenols standard (each 20 mg/L in methanol).

APPENDIX B: ESEM IMAGES

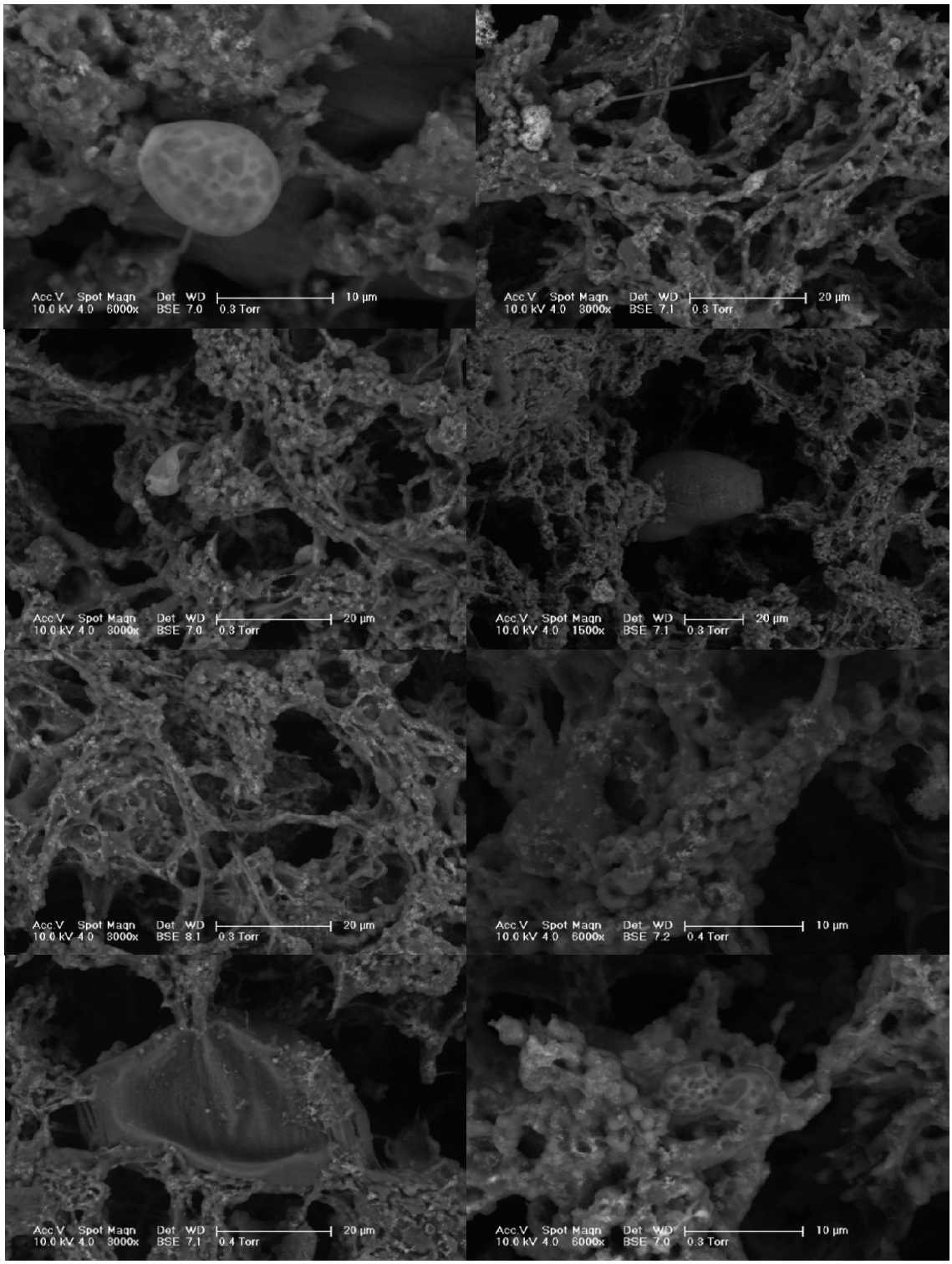


Figure B.1. Environmental scanning electron microscopy of non-acclimated activated sludge taken from the mother reactor

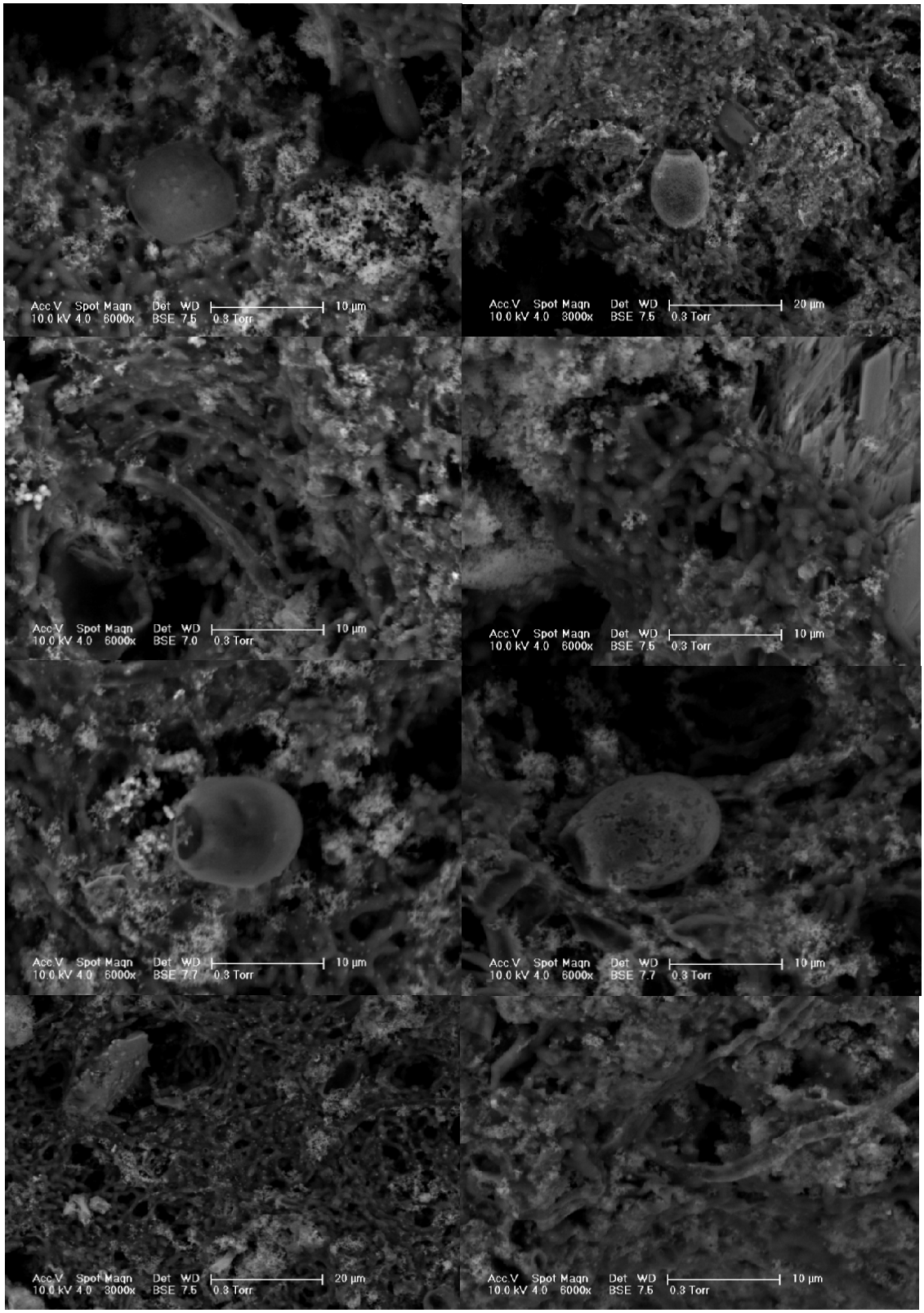


Figure B.2. Environmental scanning electron microscopy of activated sludge acclimated to phenol and 2-chlorophenol.

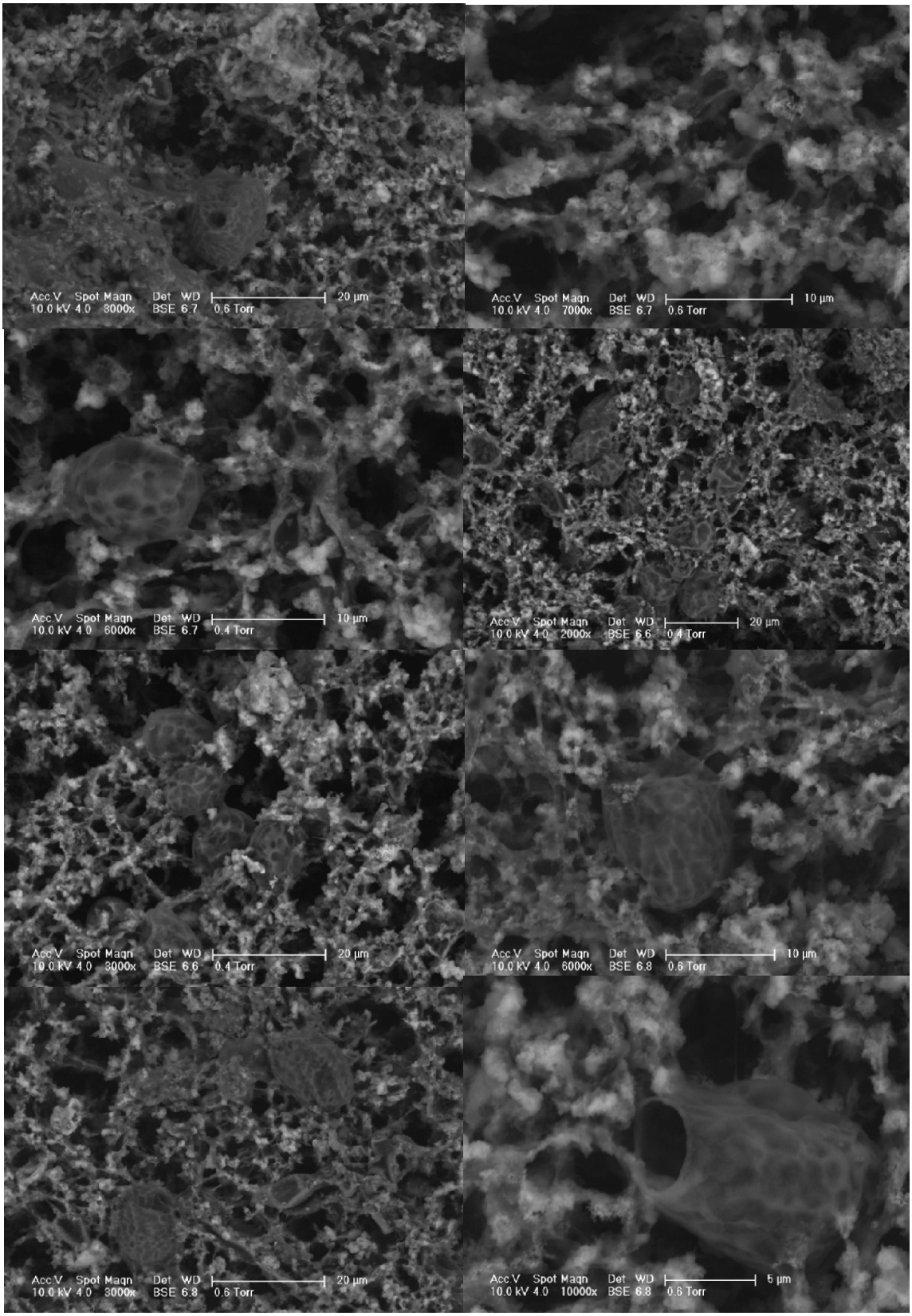


Figure B.3. Environmental scanning electron microscopy of activated sludge acclimated to phenol and 2-nitrophenol.

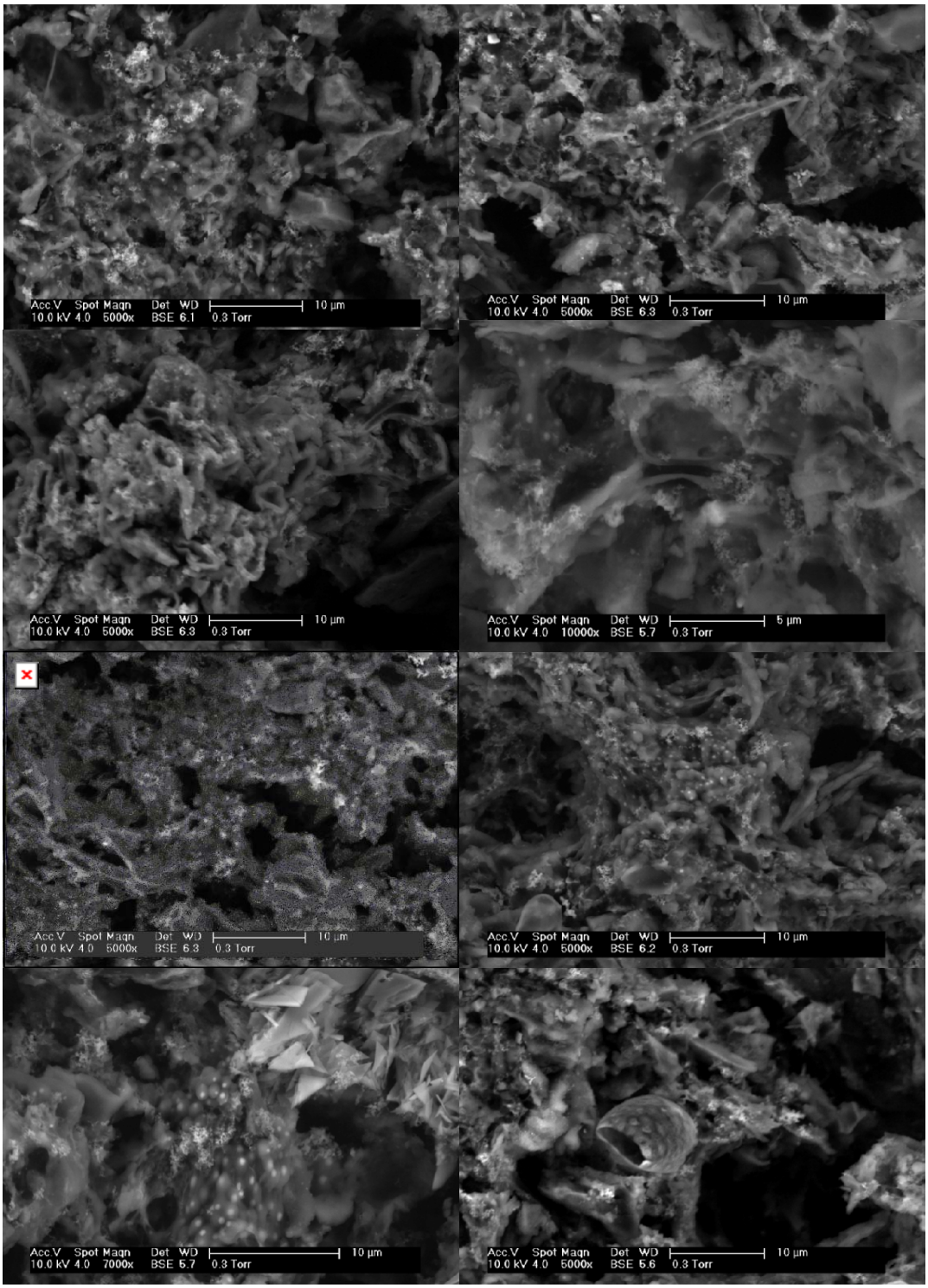


Figure B.4. Environmental scanning electron micrographs of microorganisms on SA4 carbon at t=3 days in RUN 14.

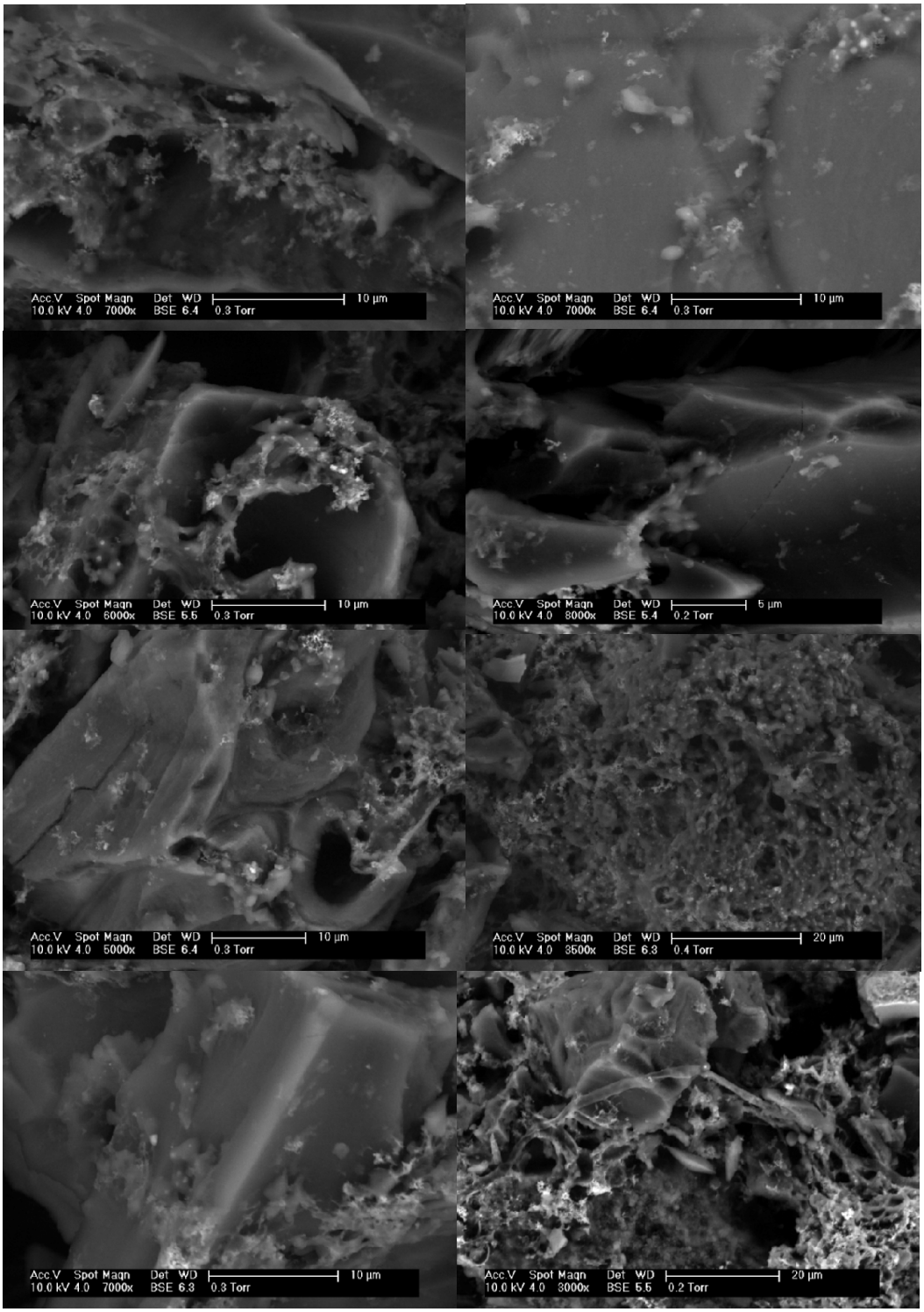


Figure B.5. Environmental scanning electron micrographs of microorganisms on CA1 carbon at t=3 days in RUN 14.

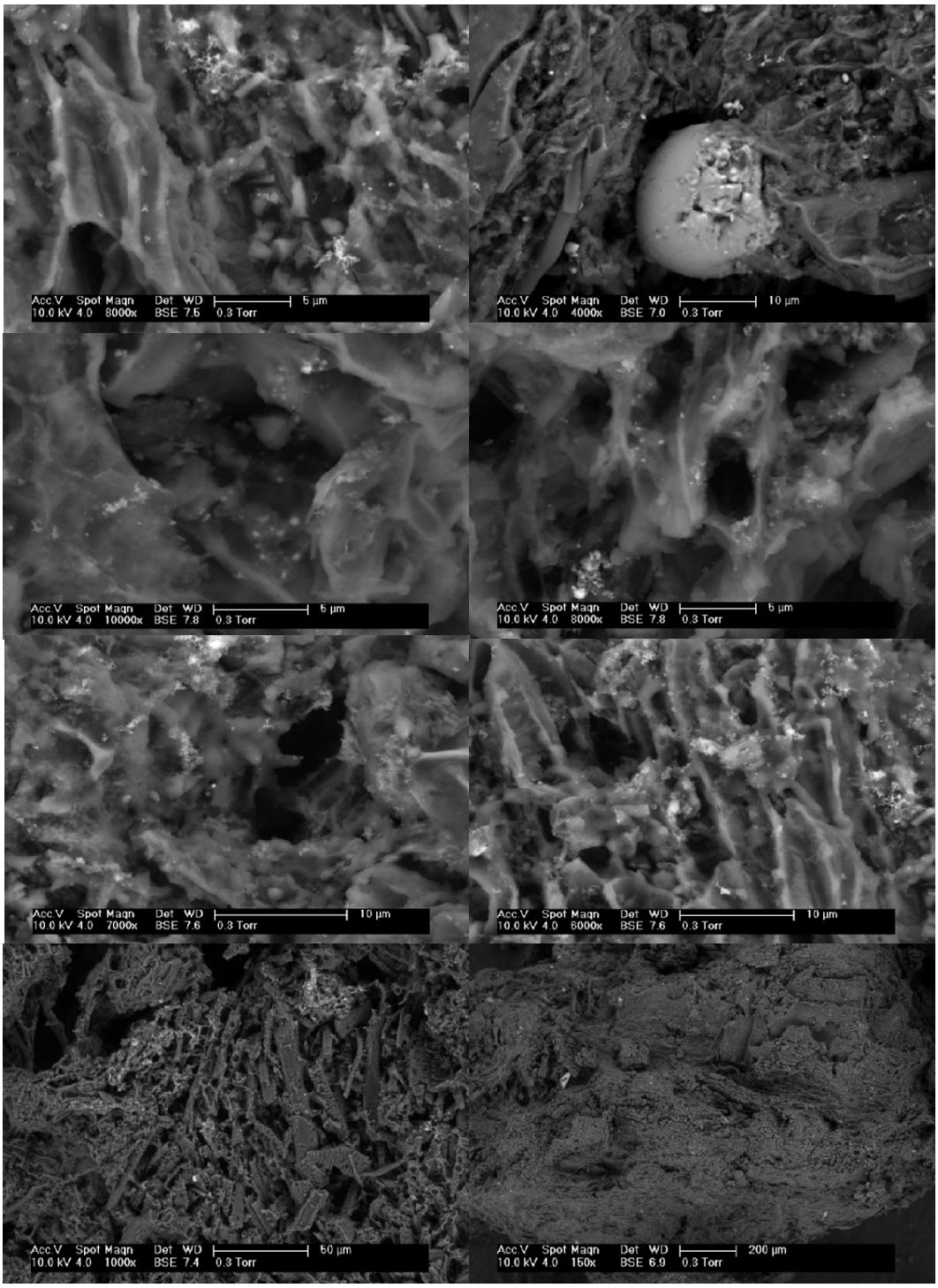


Figure B.6. Environmental scanning electron micrographs of microorganisms on PKDA carbon at t=3 days in RUN 15.

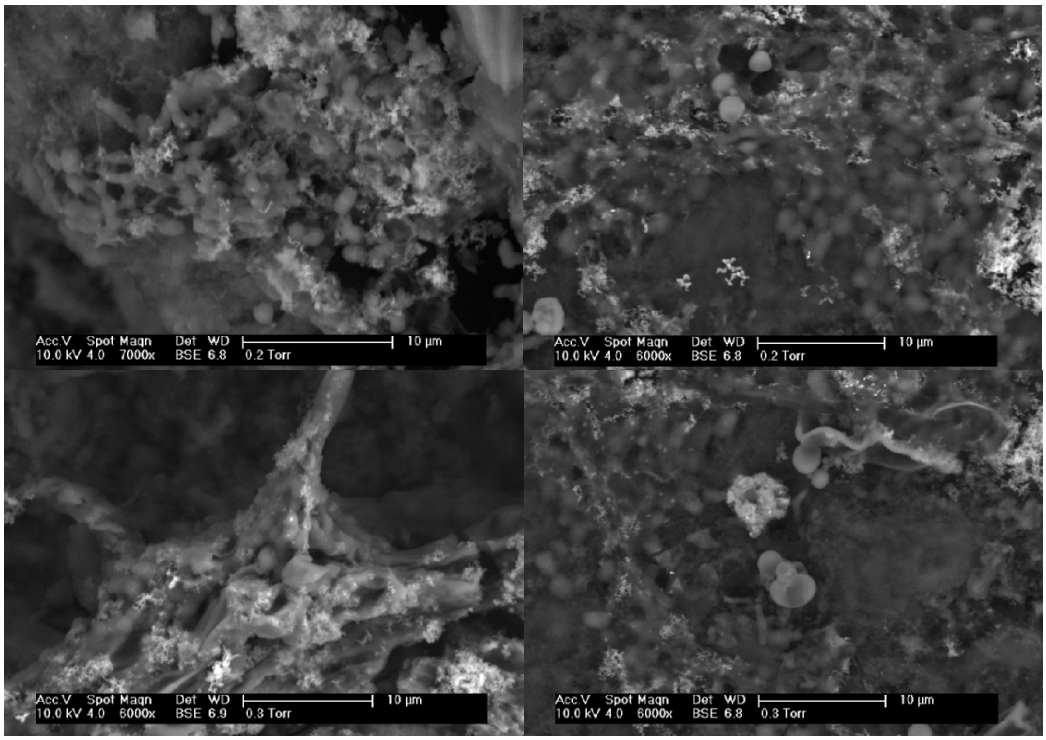


Figure B.7. Environmental scanning electron micrographs of microorganisms on CAgran at t=3 days in RUN 15.

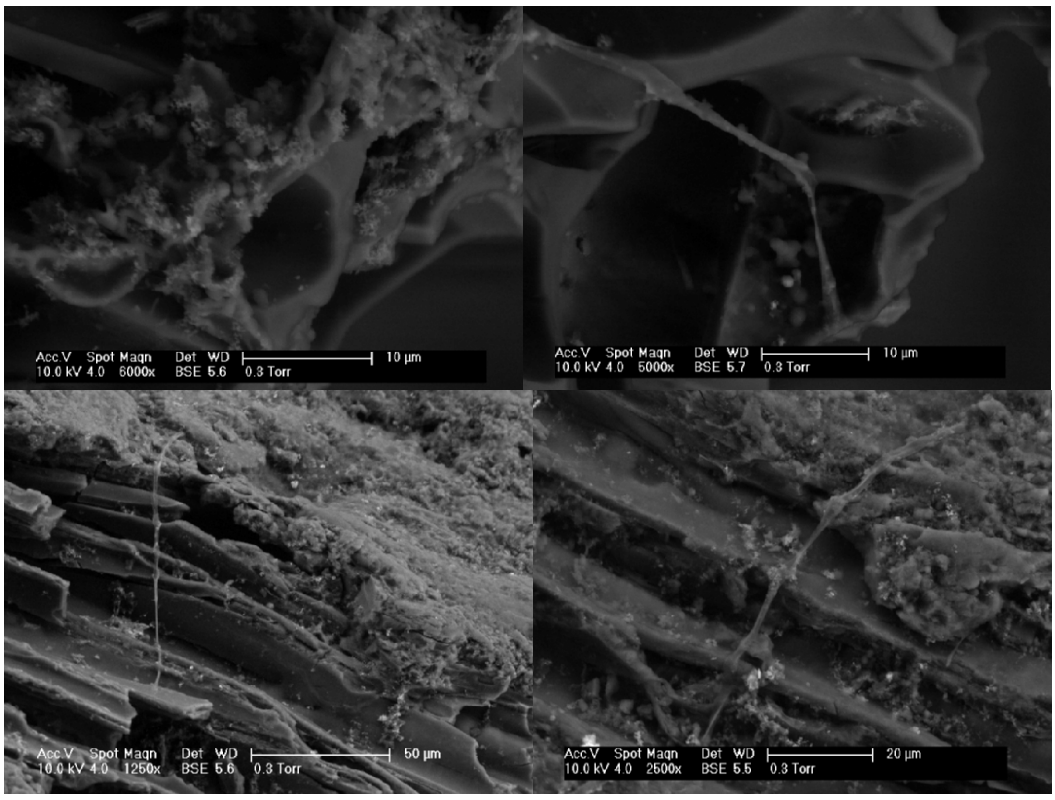


Figure B.8. Environmental scanning electron micrographs of microorganisms on CAgran at t=8 days in RUN 15.

BIOREGENERATION OF ACTIVATED CARBON IN THE TREATMENT OF PHENOLIC COMPOUNDS

Özgür Aktaş

Environmental Technology Ph.D. Thesis, 2006

Thesis Supervisor: Prof. Dr. Ferhan Çeçen

Keywords: Activated carbon, biodegradation, bioregeneration, phenolic compounds, reversibility of adsorption

This study aimed to clarify the effect of adsorbability, desorbability, biodegradability and activated carbon type on the extent of bioregeneration in the treatment of phenolic compounds in activated sludge systems combined with activated carbon. Bioregenerabilities of activated carbons preloaded with phenol, 2-chlorophenol and 2-nitrophenol were studied. For this purpose, four different activated carbon types; thermally activated and chemically activated powdered carbons (PAC), and their granular countertypes (GAC) with similar physical characteristics were used. Thermally activated carbons were better adsorbers for phenolic compounds than chemically activated ones. However, apparently higher adsorption irreversibility was calculated in the case of thermally activated carbons. The results suggested that, rather than the physical form, carbon activation type and chemical surface characteristics played a more important role on adsorbability of phenolic compounds and its reversibility. Also, adsorption and its reversibility were highly dependent on the type of the target compound. Bioregeneration of chemically activated carbons was also higher than thermally activated ones. This showed that bioregeneration was controlled by the reversibility of adsorption. The results suggested that carbon activation type was of crucial importance for

bioregeneration. PAC and GAC countertypes showed comparable bioregeneration efficiencies indicating that carbon size was not an effective factor. Oxidative polymerization of phenol and 2-chlorophenol was a plausible explanation for lesser bioregeneration of thermally activated carbons. However, bioregeneration efficiencies of thermally activated carbons were much higher than their total desorbabilities. This indicated the presence of exoenzymatic bioregeneration. Bi-solute experiments showed that competition for adsorption sites greatly determined the extent of adsorption reversibility and bioregeneration. Cometabolic biodegradation of 2-chlorophenol and 2-nitrophenol in the presence of phenol resulted in efficient bioregenerability of activated carbons, when they were loaded with non-growth substances together with a growth substrate.

FENOLİK BİLEŞİKLERİN ARITIMINDA AKTİF KARBONUN BİYOREJENERASYONU

Özgür Aktaş

Çevre Teknolojisi Doktora Tezi, 2006

Tez Danışmanı: Prof. Dr. Ferhan Çeçen

Anahtar Kelimeler: Adsorpsiyonun tersinirliği aktif karbon, biyodegradasyon, biyorejenerasyon, fenolik bileşikler,

Bu çalışmanın amacı, aktif çamur ve aktifkarbonun birarada bulunması halinde, fenolik maddelerin gideriminde, adsorplanabilirlik, desorplanabilirlik, biyolojik olarak ayrıştırılabilirliğin ve aktif karbon tipinin biyorejenerasyon mertebesine olan etkisini ortaya koymaktır. Çalışmada, fenol, 2-klorofenol ve 2-nitrofenol ile önceden yüklenmiş aktif karbonların biyorejenere edilebilirliği araştırılmıştır. Bu amaçla termal ve kimyasal olarak aktive edilmiş toz aktif karbonlar ve bunların benzer fiziksel özelliklere sahip granüler eşleri kullanılmıştır. Termal olarak aktive edilmiş karbonlar fenolik maddeleri daha iyi adsorplamışlardır. Ancak termal olarak aktive edilmiş karbonlarda adsorpsiyonun tersinirliğinin daha düşük olduğu bulunmuştur. Sonuçlar göstermiştir ki, aktif karbonun fiziki formundan ziyade, aktivasyon yöntemi ve kimyasal yüzey özellikleri, fenolik bileşiklerin adsorpsiyonunu ve bunun tersinirliğini daha fazla etkilemektedir. Aynı zamanda, adsorpsiyonun ve tersinirliğinin hedef bileşiğe göre de değiştiği bulunmuştur. Kimyasal olarak aktive edilmiş karbonlar, yüksek adsorpsiyon tersinirliklerine bağlı olarak daha fazla biyorejenerasyona uğramışlardır. Bu da biyorejenerasyonun adsorpsiyonun tersinirliğine bağlı olduğunu göstermiştir. Sonuçlar, karbon aktivasyon yönteminin biyorejenerasyon açısından

çok önemli olduğunu göstermiştir. Toz ve granüler aktif karbon eşlerinde biyorejenerasyon verimlerinin birbiriyle karşılaştırılabilir olması, partikül büyüklüğünün önemli bir etken olmadığını göstermiştir. Fenol ve 2-klorofenolün oksidatif polimerizasyonu termal olarak aktive edilmiş karbonların daha az biyorejenere olmalarına neden olmaktadır. Ancak, termal olarak aktive edilmiş karbonların biyorejenerasyon verimleri toplam desorplanabilirliklerinden fazla bulunmuştur. Bu da hücre dışındaki enzimlere bağlı bir biyorejenerasyonun varlığına işaret etmektedir. İki bileşikle yapılan deneyler göstermiştir ki, bileşiklerin adsorpsiyon için rekabet etmesi, adsorpsiyonun tersinirliğini ve biyorejenerasyonun mertebesini belirlemektedir. Fenolün varlığında, 2-klorofenol ve 2-nitrofenol kometabolik olarak ayrıştırılabilmiştir. Bu da biyolojik ayrıştırılabilirliği zor olan maddelerle yüklenmiş aktif karbonların biyolojik olarak giderilebilen maddelerin varlığında biyorejenerasyonunu mümkün kılmıştır.