

1 THE TWO-PHASE BIOREACTOR
2 WATER / SILICON - OIL : PROSPECTS IN THE
3 OFF- GAS TREATMENT

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14 KEYWORDS: TWO-PHASE BIOREACTOR ; SILICON OIL; V.O.C.;

15 OFF GAS TREATMENT

16 ABSTRACT: A research was carried out to develop a biphasic biological

17 reactor able to clean the gas effluents polluted by volatile organic compounds

18 (V.O.C). Initially, *Rhodococcus erythropolis* T 902.1 had been selected on the

19 basis of its capacity to degrade isopropyl-benzene (IPB).

20 The effect of gas flow and IPB concentration on the biodegradation of IPB was
21 evaluated.

22 The results show that the use of silicon-oil allows large quantities of IPB to be
23 absorbed within the medium of biological abatement. On the other hand, the
24 biodegradation rate is directly correlated to the inlet flow of IPB. Thus, the
25 reactor presents interesting opportunities in the biological treatment of gas
26 effluents.

27 1 INTRODUCTION

28 A research is carried out within the framework of gaseous treatment. It aims to
29 develop a biphasic reactor "water / silicon- oil ". Silicon-oil is used to allow a
30 better biological abatement of the aromatic organic compounds by improving
31 their solubility within the two-phase bioreactor. Initially a bacterial strain
32 (*Rhodococcus erythropolis* T 902.1) was selected on the basis of its good
33 capacity to degrade isopropyl-benzene (IPB), a compound selected as a model
34 for the family of benzene. Various research was performed in order to improve
35 degradation of VOC in gas effluents, particularly by improving the mass
36 transfer of VOC within the reactor. Yeom and Daugulis (2001) (1) recommend
37 the use of a biphasic biological reactor whose organic phase (hexadecane)
38 constitutes 1/3 of the reactional medium. Hexadecane presents the property to
39 be slightly toxic for the micro-organisms. A similar process showed its
40 effectiveness on the biodegradation of a mixture of organic pollutants
41 (B.T.E.X.) by a *Pseudomonas* sp. strain (2a ; 2b).

42 Van Ede *et al.* (1995) (3) showed, by the theory called Film Variable Holdup
43 (F.V.H.) model, an enhancement in the gas transfer thanks to the presence of a
44 dispersed octene phase.

45 Moreover, it could be shown that the transfer rate and the biodegradation of
46 apolar pollutants in biological waste gas treatment, as well as transfer rate of
47 oxygen, can be enhanced by a dispersed organic solvent (FC40, 10 % V/V).
48 Theoretically, it could be shown that the addition of solvent has a more
49 significant effect on the enhancement of transfer rate in case of poorly water-
50 soluble compounds compared to moderately water-soluble ones. (Teresa *et al.*,
51 1997) (4).

52 On the other hand, Nielsen *et al.*(2003) (5) showed an increase of the oxygen's
53 solubility within the system by the addition of hexadecane. This increase
54 provides a potential for enhancement of oxygen's mass transfer rate.

55 Dumont and Delmas (2001) (6) reviewed the general concept of oil-in-water
56 systems. This was supposed to demonstrate the ability of an immiscible oil
57 phase to influence the possible way for transfer from the gas phase to the
58 aqueous phase.

59 An other way to enhance oxygen gas transfer rate is proposed by using
60 soybean oil-in-water dispersion (7).

61 In this context, silicon oil can also be used to reproduce the effect of a solvent.
62 Thus, Budwill and Coleman (1997) (8) developed a biofilter with silicon oil
63 addition that improves hexane biodegradation. Moreover, Gardin *et al.*

64 improved the biodegradation of xylene and butyl acetate by using an aqueous-
65 silicon oil two-phase system.

66 Finally, Aldric (2001) (9) showed the value of the use of the silicon oil in a
67 proportion of 10 % in a two-phase bioreactor. Silicon oil allows an important
68 improvement of the gas retention into biphasic medium, i.e. the volume of gas
69 retained within a volume of reactional medium liquid. Furthermore,
70 coefficient of oxygen mass transfer is not decreased compared to an aqueous
71 medium. These aspects show the possibilities of application in the field of the
72 off-gas treatment. Indeed, the silicon oil allows a significant solubilisation of
73 volatile organic compounds. The object of this study thus consists in
74 specifying the influence of silicon oil on the biodegradation of IPB, selected as
75 model for the B.T.E.X. compounds.

76 2 MATERIALS AND METHODS

77 2.1 *Strain and chemicals*

78

79 The *Rhodococcus erythropolis* strain was obtained from the collection of the
80 Walloon Center for Industrial Biology (C.W.B.I.; Belgium) (10 and 11).

81 All substrates and other chemicals were purchased from VWR international
82 (Leuven, Belgium) or Aldrich (Bornem, Belgium).

83 2.2 *Bioreactor and assembly*

84 The stirred bioreactor used for biodegradation (LSL Biolafitte BL06.1, Saint
85 Germain en Laye, France) was described by Aldric (2001). Its reactional

86 volume reaches 4.5l and the stirring speed was maintained at 600 rpm. The
87 assembly is schematized in figure 1.

88 The IPB concentrated gas is generated by stripping within a thermostated
89 glass bottle. The gas flow is permanently controlled by a flow meter.

90 The concentration of IPB in the gas coming in the bioreactor is controlled by
91 an adjustable mixture between polluted gas and air.

92 2.3 *Experimental design*

93 Doehlert design (12) was selected to evaluate the effect of two factors on the
94 biodegradation: gas flow and IPB concentration of inlet gas. The values of
95 concentration and flow corresponding to the experimental points are selected
96 so that the pairs concentration-flow are located at the angles of a perfect
97 hexagon in bidimensional space "Concentration / Flow". Nevertheless, the
98 adjustment of the parameters to their fixed values is difficult because of the
99 technical constraints. The statistical analysis of the results thus requires to take
100 into account the real average values of the two studied parameters. The shifted
101 points obtained in experiments are indicated on figure 9. The area defined by
102 the Doehlert design is a circle in a two dimensional space. 5 levels were
103 retained (mg/Nm^3 *) for the "concentration" factor and 3 levels for factor
104 "flow" (l/min).

105 The 10 performed experiments are included and characterized in table 1.
106 Shaded experiments represent the repetitions of the central experiment.

* Nm^3 : m^3 under normal conditions of temperature and pressure (25°C – 101325 Pa)

107 Statistical analysis of the results was carried out with the software SAS ® .
108 The Doehlert design (12) permits to establish a second degree regression
109 model with interactions of the second order

110 2.4 *Sampling and analytical methods*

111

112 Gas samples are regularly taken from each bubble of sampling as well as in
113 the liquid reactional medium. IPB concentration was estimated thanks to a
114 Perkin Elmer headspace sampler HS 40 XL (for liquid samples) and a gaz
115 chromatograph Hewlett Packard 5890 equipped with a Alltech INC. Deerfield
116 EC-WAX column and flame ionisation detector (for gas-samples).
117 Temperatures of the injector, column and detector were respectively 153, 150
118 and 250 °C.

119 2.5 *Implementation of the biomass*

120 The culture of *Rhodococcus erythropolis* in 868 medium (glucose 20g/l;
121 casein peptone 20 g/l; yeast extract 10 g/l) is harvested after 64 hours (optical
122 density 600 nm =1.4). The inoculum for the biological reactor is obtained by
123 centrifugation of 2.25 L of this culture. The pellet obtained is washed twice
124 and diluted in 200 ml saline water (6g/l NaCl). The inoculum is then
125 introduced into the bioreactor where the medium for biodegradation is
126 composed of silicon oil (10% V/V) and aqueous medium M284 (90 % V/V)
127 whose composition is : Tris-HCl 6.06g/l ; NaCl 4.68g/l ; KCl 1.49g/l; NH₄Cl

128 1.07g/l ; Na₂SO₄ 0.43g/l; MgCl₂. 6H₂O 0.20g/l ; Na₂HPO₄ 2H₂O 40mg/l ;
 129 CaCl₂. 2H₂O 30mg/l ; Fe(III)NH₄citrate 4.8mg/l. ZnSO₄.7 H₂O 0.144 mg/l ;
 130 MnCl₂.4 H₂O 0.1 mg/l ; H₃BO₃ 0.062 mg/l ; CoCl₂.6 H₂O 0.19 mg/l; CuCl₂.2
 131 H₂O 0.017 mg/l ; NiCl₂.6 H₂O 0.024 mg/l ; Na₂MoO₄.2 H₂O 0.036 mg/l;
 132 ethanol 1g/l.

133 2.6 *Determination of concentrations and flows*

134
 135 Only the data corresponding to a stabilization of the IPB concentration within
 136 the liquid medium is retained. The following relation is indeed correct because
 137 IPB consumption by the biomass (term to the left) is equal to the transferred
 138 quantity (term to the right):

$$139 \quad (Q_{in} - Q_{out}) = K_L a (C_L^0 - C_L)$$

140 where K_La is the total coefficient of mass transfer for IPB (min⁻¹); Q_{in} and Q_{out}
 141 are respectively the inlet flow and outlet flow of IPB (mg IPB/min.l of
 142 reactional medium); C_L⁰ = saturating concentration of IPB in the biphasic
 143 liquid medium (mg/Nm³) ; C_L = equilibrium concentration of IPB in the
 144 biphasic liquid medium (mg/l).

145 Inlet flow and outlet flow of IPB are given by the following equations:

146

147

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$$Q_{IN} = \frac{Conc.IN_{mean} \times flow}{vol}$$

150

$$Q_{OUT} = \frac{Conc.OUT_{mean} \times flow}{vol}$$

$$Conc_{mean} = \sum_{i=1}^n \frac{Conc_i \times (t_i - t_{i-1})}{t_{tot}}$$

151

152 where $Conc_{mean}$ = weighted average of concentrations during an equilibrium
153 phase (mg/Nm³).

154 $Conc_i$ = specific concentration (mg/Nm³) measured by gas injection at time
155 $t=i$.; flow = flow of gas effluent loaded in IPB; t = time (h) and Vol= the
156 volume of the reactional medium.

157 2.7 Determination of the biodegradation rate

158

159 The biodegradation rate can be easily calculated by the following formula:

160 Biodegradation rate = 100 x ($Q_{in} - Q_{out}$) / Q_{in} where Q_{in} and Q_{out} are described
161 above.

162

163

164 3 RESULTS AND DISCUSSION

165 3.1 *Follow-up of the IPB biodegradation in a gas effluent*

166
167 Previously, *Rhodococcus erythropolis* T 902.1 showed good potential for
168 biodegradation of aromatic compounds (benzene, toluene and xylene) in liquid
169 medium. Moreover, this strain has very interesting properties to produce
170 starter culturess, specifically due to drying procedure. The industrial
171 applications are thus possible (10).

172 More specifically, this strain contains a catabolic plasmid conferring it the
173 aptitude to degrade toluene and the other aromatic compounds such as the
174 IPB, selected as model within the framework of this research (11).

175 In this research, this strain was implemented in a two-phase bioreactor which
176 was developed to degrade IPB in gas effluents. The use of the silicon oil is the
177 originality of the proposed two-phase reactor. This phase is used to improve
178 the transfer and the biodegradation of the IPB from off-gas.

179 A laboratory scaled two-phase reactor was developed to study the influence of
180 the IPB concentration and the flow of gas (fig.1).

181 The device permits generation of a polluted effluent with a flow and a
182 specified IPB concentration. This device is followed by sample points within
183 the reactional liquid medium, at the entry and the exit of the bioreactor (see
184 description, fig. 1). The follow-up makes it possible to determine the rate of
185 IPB biodegradation and the loading capacity of the bioreactor for the various
186 values of the studied parameters (flow and IPB concentration). Moreover, in

187 order to follow the two studied parameters, Doelhart design was implemented
188 as explained previously (see paragraph 2.3). The experiments included in
189 Doelhart design were monitored during 3 days. As described previously, the
190 measurements taken into account to determine the parameters (biodegradation
191 rate and loading capacity) are those corresponding to a stability of the
192 concentration in the liquid medium (consumed quantity = transferred quantity)
193 The evolution of IPB concentration in inlet gas, outlet gas and within the
194 liquid medium is given, as example, in figures 2 and 3 for the two most
195 significant experiments corresponding respectively to a low flow and a high
196 flow of IPB (mg/min.l)

197 Figure 2 shows the evolution of the IPB concentration as described above. The
198 experiment presented at figure 2 corresponds to a 0.60 mg/min.l IPB flow of
199 reactional medium. Both concentration within the liquid and concentration in
200 outlet gas of bioreactor are very low, what represents a very good
201 biodegradation yield.

202 The experiment presented at figure 3 corresponds to the highest loading of IPB
203 (9.47 mg/min.L) and shows the evolution of the IPB concentration for this
204 experiment. It is obvious that the concentration in outlet gas is high (1727
205 mg/Nm³ on average) but comparatively to concentration in inlet gas and to the
206 gas flow, the biodegradation yield remains very acceptable.

207 These two examples show that the IPB biodegradation by the selected strain is
208 important, including for high loads of IPB (more than 9 mg/min.l ; 540g/m³.h).

209 In order to define the potentialities of the proposed bioreactor, the
210 biodegradation rate (% of IPB degraded by the biomass) and the loading
211 capacity (mg/min.l) were given for each experiment.

212 3.2 *Biodegradation rate functions of flow and concentration*

213
214 The biodegradation rate is the relationship between the quantity of pollutant
215 degraded by the biomass (mg/min.l) and the flow of pollutant entering
216 bioreactor (mg/min.l). It expresses the efficiency of the bioreactor for each
217 pair (concentration - flow).

218 For each experiment of Doehlert design, the biodegradation rate of the IPB
219 was calculated as indicated below:

$$220 \quad \Gamma = \frac{100 \times Q_{in} - Q_{out}}{Q_{in}}$$

221 where Γ is the biodegradation rate of IPB (%).

222 Q_{in} and Q_{out} are respectively the flows of IPB entering and outgoing from the
223 bioreactor (mg/min.l). The biodegradation rate of IPB for each experiment and
224 each day is shown in figure 4. The biodegradation rates are high in all
225 experiments, except for experiments 5 and 7. In the implemented range of
226 flow and concentration, the biodegradation rate is never lower than 53 %
227 except for the first day of experiment 8. After comparing the biodegradation
228 rate according to the days of experimentation, it is observed that the
229 biodegradation rate is often the highest at the end of the experiment (third

230 day). This can be explained by an adaptation phase of the biomass to the
231 presence of IPB.

232 As described previously, the objective of this research is to evaluate the effect
233 of the gas flow and the IPB concentration on the effectiveness of the proposed
234 bioreactor, i.e. on the biodegradation rate. It is why a statistical analysis of the
235 results was carried out.

236 3.3 *Statistical analysis of the results*

237

238 The statistical analysis of the results, according to the response surface
239 method, is carried out in order to establish a second degree regression model
240 between the biodegradation rate and the two chosen parameters: gas flow
241 (l/min.) and IPB concentration (mg/Nm³).

242 Considering that the cells must be adapted, only the values of biodegradation
243 rate corresponding to the third day are taken into account for the statistical
244 analysis.

245 For this purpose, the values of biodegradation rate measured were transformed
246 by the arcsinus square root transformation. This traditional transformation
247 permits to observe the conditions for application for the analysis of the results
248 according to Doelhart (12). On the basis of above mentioned considerations, a
249 second degree regression model can be proposed:

$$250 \arcsin^{-1} \Gamma = 1.987 - 0.216Q - 1.4 * 10^{-4} Conc_{in} + 1.12 * 10^{-4} Q \times Conc_{in} + 9.97 * 10^{-3} Q^2 + 1.29 * 10^{-8} Conc_{in}^2$$

251

$$252 R^2 = 0.9399$$

253 Γ represents the IPB biodegradation rate (%)
 254 Q is the inlet flow of gas (l/min) - first factor.
 255 Conc_{in} is the IPB concentration of inlet flow (mg/Nm^3) - second factor.
 256 For each estimated coefficient, table 2 presents p value. As shown in table 2,
 257 the first degree coefficient of the flow (0.0017) is highly significant. The
 258 factor flow (Q) is thus most influential on the biodegradation rate. In addition,
 259 figure 5 permits visualization of the combined effect of flow and concentration
 260 of the effluent on the biodegradation of the IPB. The contours of second order
 261 equation indicate the "concentration-flow" pairs for which the same
 262 biodegradation rate is observed. By means of this diagram, it is possible to
 263 estimate the biodegradation rate for a flow-concentration pair. The shape of
 264 the diagram indicates that the variation of the flow induces a strong variation
 265 of the biodegradation rate. On the other hand, for the same flow, the variation
 266 of effluent concentration slightly influences the biodegradation rate.
 267 These observations can be explained by the fact that the two-phase bioreactor
 268 is able to transfer and absorb great quantities of pollutants only if the
 269 superficial gas velocity, generated by a high flow, is not too high into the
 270 bioreactor.

271 3.4 *Loading capacity for the experiments*

272
 273 The biodegradation rate characterizes the efficiency of a process. However, it
 274 is also important to determine the limits of the bioreactor. Therefore, the
 275 loading capacity can be defined as the absolute quantity of IPB degraded by

276 the biomass per unit of time and unit of reactional volume: $Q_0 = Q_{in} - Q_{out}$
277 (mg/min.l). Figure 6 presents the described loading capacity. The loading
278 capacity is logically correlated with the flow of IPB from inlet gas (mg/min.l).
279 However, the limit seems to be reached in experiment 5 with a 7.5 mg/min.l.
280 loading capacity (450g/m³.h). This limit can be explained by figure 7 which
281 shows the IPB concentration in the biphasic liquid medium. Experiments 5, 7
282 and 9 (fig. 7) show that the concentration in the liquid medium reaches
283 approximately 1200 mg/l. This concentration within the liquid medium (C_L)
284 seems to be a factor limiting the mass transfer of the IPB. Indeed, when the C_L
285 concentration increases, the potential of transfer ($C_L^0 - C_L$) decreases.

286 3.5 *Evolution of cellular concentration in the bioreactor*

287 The initial cellular concentration within the bioreactor is standardized.
288 However, the evolution of the biomass is variable from one experiment to
289 another. It is thus important to discuss the influence of the cell multiplication
290 on the effectiveness of the biodegradation. The evolution of cellular
291 concentrations is presented in figures 8 (four repetitions of the central
292 experiment) and 9 (other individual experiments).

293 The variability of observed growth for a repetition of the central experiment
294 (fig.8) does not permit to highlight a relation between the quantity of brought
295 pollutant per unit of time and the cellular multiplication. However and
296 generally, an adaptation phase of microorganisms is observed at the beginning
297 of the experiment (24h). The cellular concentration increases then slightly

298 during experiments. This observation must be coordinated to the increase of
299 the degradation rate functions of time. It should be noted that the cell
300 multiplication is minimal for experiments corresponding to the lowest flows of
301 IPB (respectively 0.57 and 0.11 mg/min.l; exp. 2 and 8). For small quantities
302 of added carbon substrates, the cell multiplication is then very low. On the
303 other hand, growth is observed during the experiments with higher potential
304 carbon sources.

305 In addition, the quadruple repetition of the central experiment (fig.8) allows to
306 compare 4 identical experiments and to show that the most significant
307 biodegradation rate (82%; experiment 3) is obtained when the cell
308 multiplication was the highest. The cellular concentration thus plays a role in
309 the effectiveness of the bioreactor.

310 *3.6 Conclusions*

311 The obtained results show that the use of a two-phase bioreactor with silicon-
312 oil as second phase allows absorption of large quantities of IPB and its good
313 biological degradation with appropriate microorganism. The analysis of the
314 results shows that the biodegradation rate seems to be related mainly to the gas
315 flow (l/min.) comparatively to the IPB concentration (mg/Nm³) in the gas
316 effluent.

317 A limit of concentration in the liquid medium can also be suggested, what
318 leads to a transfer limitation of the IPB from polluted gas towards the liquid
319 medium by a reduction in the potential of transfer ($C_L^0 - C_L$). Otherwise, it was

320 shown that an increase in the cellular concentration during experiment
321 improves the effectiveness of the bioreactor.

322 Thus, in the tested configuration, the two-phase bioreactor is able to degrade
323 7.5 mg/min.l of reactional media ($450\text{g/m}^3\cdot\text{h}$).

324 It is also shown that an adaptation step of the biomass is necessary to reach
325 substantial rates of abatement. However, the biomass is maintained and even
326 increases during the experimentation.

327 The bioscrubbers usually used in the off-gas treatment are able to treat a
328 polluted effluent concentrated with 1000 mg/Nm^3 at a flow of 1.5 l/min.l
329 ($90\text{m}^3/\text{m}^3\cdot\text{h}$). This corresponds to a flow of 1.5mg/min.l of bioscrubber (13).

330 The proposed reactor presents interesting opportunities in the biological
331 treatment of gas effluents polluted by aromatic compounds in high
332 concentration. The suggested process might be applied in the range of
333 concentration and flow where thermal oxidation is too expensive (between 1
334 and 7 g/Nm^3).

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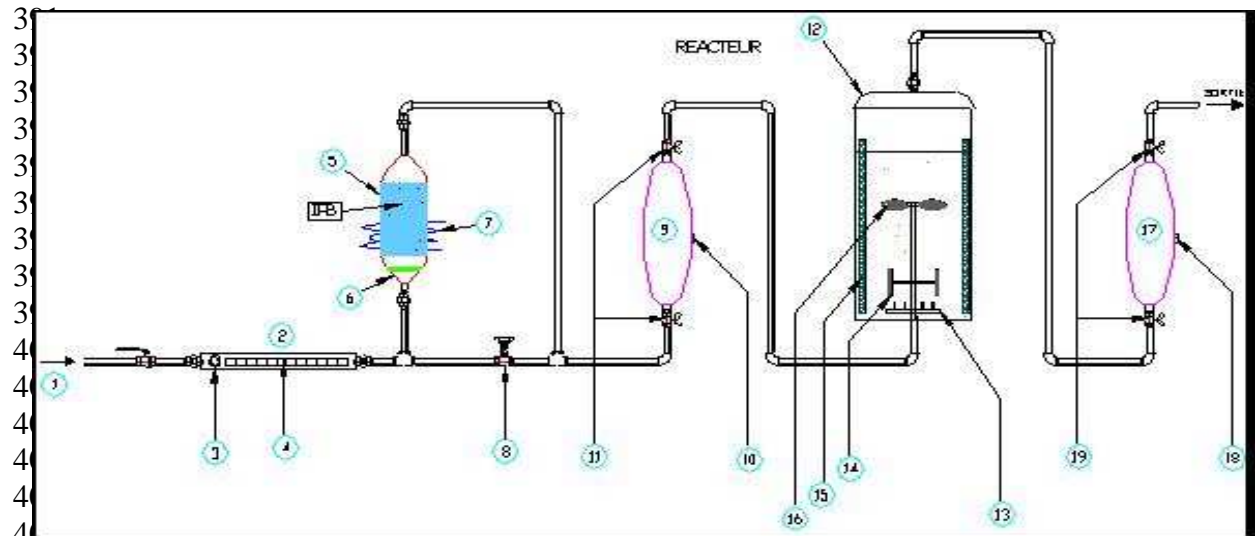
386 Figures

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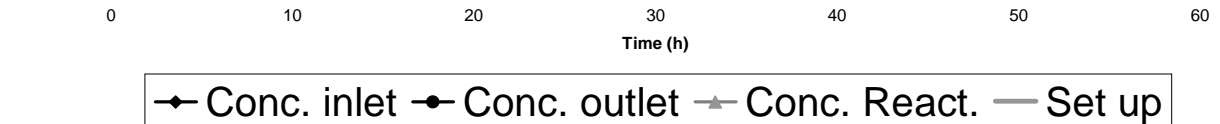
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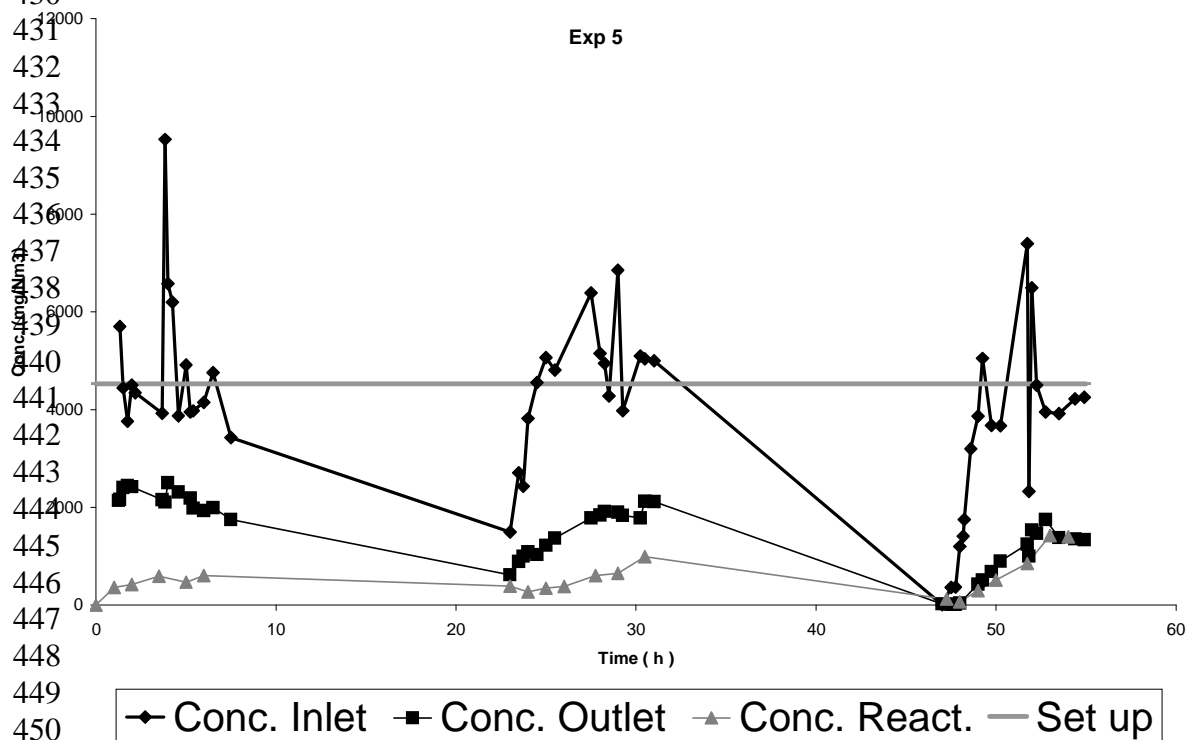
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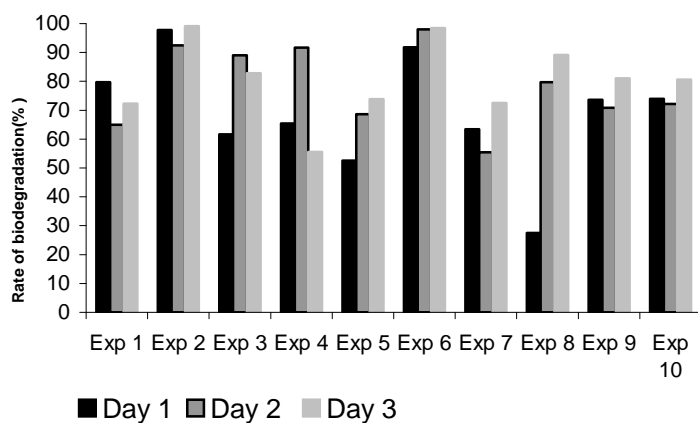
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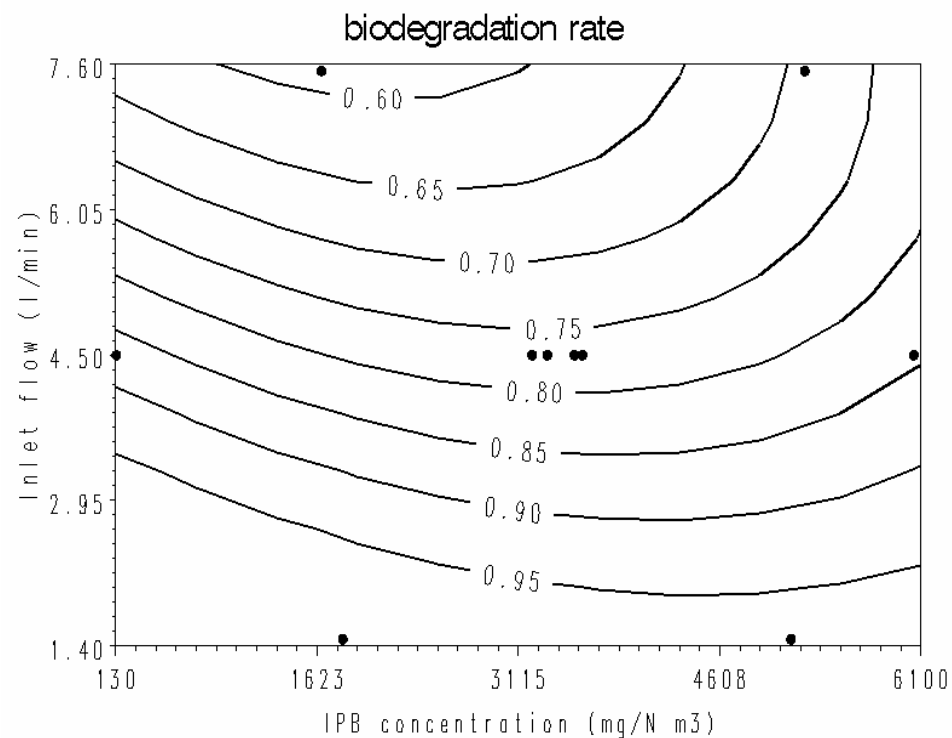
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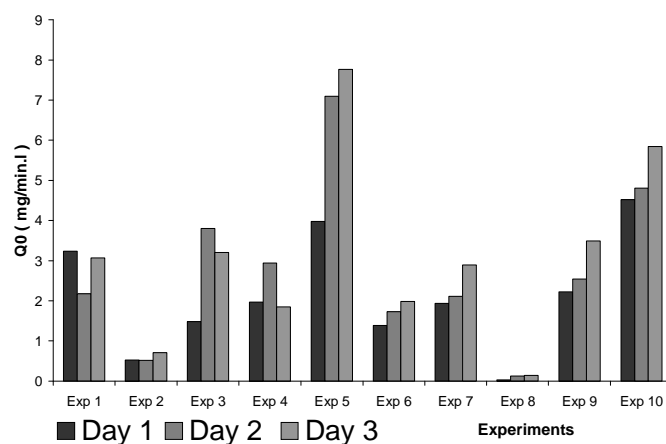
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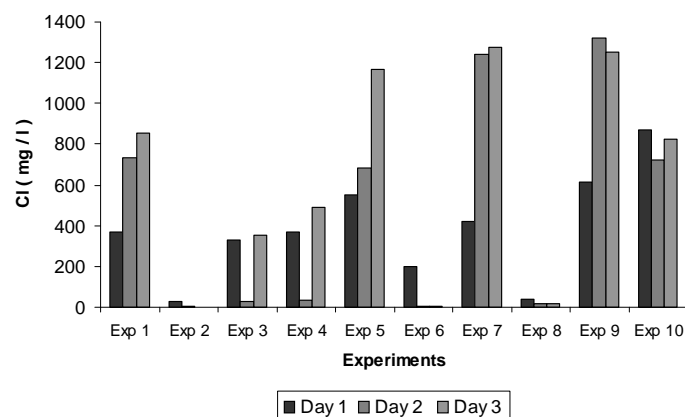
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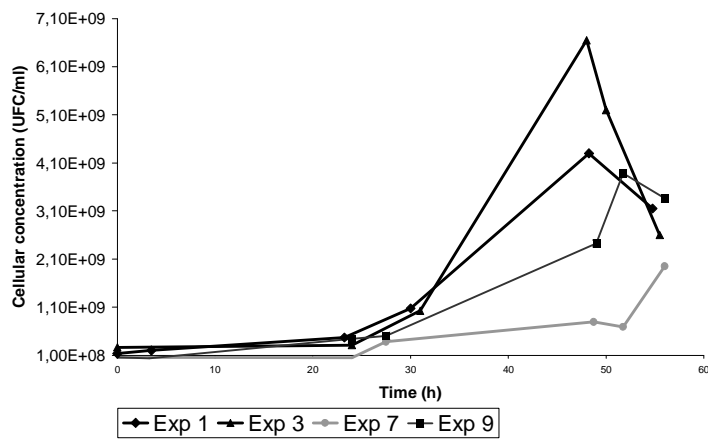
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