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## Transfer and degradation of polyacrylamide based flocculants in hydrosystems: a review

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1 **Title: Transfer and degradation of polyacrylamide based flocculants in hydrosystems: a review**

2

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11

12 **Abstract**

13 The aim of this review was to summarize information and scientific data from the literature dedicated  
14 to the fate of PAM-based flocculants in hydrosystems. Flocculants, usually composed of  
15 polyacrylamides (PAMs), are widely used in several industrial fields, particularly in minerals extraction,  
16 to enhance solid/liquid separation in water containing suspended matter. These polymers can contain  
17 residual monomer of acrylamide (AMD) which is known to be a toxic compound. This review focuses  
18 on the mechanisms of transfer and degradation, which can affect both PAM and residual AMD, with a  
19 special attention given to the potential release of AMD during PAM degradation. Due to PAM ability to  
20 adsorb onto mineral particles, its transport in surface water, groundwater and soils is rather limited and  
21 restricted to specific conditions. PAM can be also subject of biodegradation, photodegradation and  
22 mechanical degradation but most of the studies report slow degradation rates without AMD release. In  
23 the contrary, the adsorption of acrylamide onto particles is very low, which could favor its transfer in  
24 surface waters and ground water. However AMD transfer is likely to be limited by quick microbial  
25 degradation.

26

27 **Key words:** flocculation, polyacrylamide, acrylamide, environmental hazards, transfer, adsorption,  
28 photodegradation, biodegradation, amidase

29

## 30 1. Introduction

31 Flocculants are widely used in several industrial fields (mineral extraction, chemical industry, food  
32 processing industry, treatment of drinking water, etc.) to enhance solid/liquid separation in water  
33 containing suspended matter (Heath *et al.*, 2006, Harford *et al.*, 2011). In most cases, flocculants are  
34 polymers composed of polyacrylamide (PAM) (Taylor *et al.*, 2002). PAM is a polymer with high  
35 molecular weight, synthesized from acrylamide (AMD) and acrylic acid/acrylate (AA) (Scott *et al.*, 1996;  
36 Caulfield *et al.*, 2002). The use of flocculants enables to increase the recycling rate of process water  
37 and to decrease sludge volumes and thus the surface of settling ponds. Those ponds are open  
38 systems with possible exchanges into the near aquatic environment (water percolation towards soils  
39 and aquifers, water seepages in natural streams or rivers...), which can lead to the dissemination of  
40 flocculants into surface and groundwaters (de Rosemond & Liber, 2004; Liber *et al.*, 2005). The  
41 question of flocculants safety is now arising as a new key-environmental issue. The potential  
42 environmental hazard does not concern the polyacrylamide itself, which is generally considered as not  
43 toxic (Andersen, 2005), but the products of PAM degradation and the residual acrylamide contained in  
44 the flocculants due to incomplete polymerization process (Labahn *et al.*, 2010; Young *et al.*, 2007;  
45 Caulfield *et al.*, 2002). AMD is reported to be a neurotoxin to humans (McCollister *et al.*, 1964). AMD is  
46 also classified as a carcinogenic (level 2), mutagenic (level 2) and reprotoxic (level 3) compound  
47 (World Health Organization 1985; Molak, 1991). This review focuses on the fate of PAM-based  
48 flocculants in hydrosystems, with a major emphasis on the mechanisms of transfer and degradation  
49 which can affect both PAM and associated AMD. The section dedicated to transfer phenomenon will  
50 especially examine transport and reactivity with solid matrices. In the section devoted to degradation,  
51 abiotic and biotic factors will be discussed with a specific emphasis on potential hazardous AMD  
52 release during PAM degradation.

53

## 54 2. Polyacrylamide and acrylamide in flocculation processes and potential environmental 55 hazards

56 The word polyacrylamide and the acronym "PAM" (for polyacrylamide) refer to a group of water  
57 soluble molecules formed by polymerization of acrylamide monomers. PAMs have been used for  
58 decades in a variety of industries and have proven particularly effective in facilitating the solid-liquid  
59 separation for waste and drinking water treatment as well as for the clarification of various types of

60 effluents. High molecular weight PAMs ( $10^6$  to  $10^7$  g.mol<sup>-1</sup>) work as flocculants by causing suspended  
61 particles to bind together to form larger aggregates which can settle out quickly from solution leaving a  
62 clear supernatant (Heath *et al.*, 2006). By increasing solid-liquid separation, PAMs improve water  
63 clarification processes and water quality prior to discharge, re-use or disposal. In France, for instance,  
64 the consumption of PAM-based flocculants is about 15 kt per year. In particular, the aggregate  
65 production uses about 900 t/y of PAM-based flocculants (i.e. 6% of the total consumption) as  
66 clarification aid (UNPG 2013).

67 High molecular weight PAMs can be modified to develop non-ionic, anionic, or cationic properties for  
68 specific uses (Scott *et al.*, 1996). However for environmental applications which are described in this  
69 article, anionic PAMs are preferred because of their extremely low aquatic toxicity compared to  
70 cationic forms (Hamilton *et al.*, 1994; de Rosemond & Liber, 2004; Liber *et al.*, 2005). Common anionic  
71 PAMs are usually produced by copolymerization of acrylamide and acrylic acid or one of its salts (see  
72 Fig.1). Their molecular weights vary between  $3 \cdot 10^6$  and  $2 \cdot 10^7$  Da. The most polyvalent anionic PAM  
73 contains around 30% of acrylate (in mol).

74 Anionic PAMs used in flocculation applications are water-soluble and have little if any cross-linking.  
75 The molecules, when dissolved in water, are nominally linear, although they may be coiled or curled to  
76 varying degree due to either substitution along the chain or as a result of electrolytes in the solvating  
77 water (Sokja *et al.* 2007).

78 PAMs can be manufactured to have different molecular weights and charge densities by varying the  
79 reaction parameters and/or the relative quantities of reagents used. The charge density of PAM is  
80 often expressed as percent anionic, referring to the percentage of its monomers that contain a  
81 charged functional group. The variations can result in significant differences in the extent to which  
82 PAM will bind to different types of particles, and thus influence the flocculation efficiency. Even among  
83 products that contain anionic PAM as their active ingredient, performance can vary substantially if the  
84 PAM charge densities or molecular weights are different.

85 One of the primary areas of concern associated with the use of PAMs is the presence of residual  
86 monomer of AMD in the polymer product, due to incomplete polymerization process. AMD is solid at  
87 room temperature, highly soluble in water, has a low potential to partition to organic matter, and has a  
88 low volatilization potential in water. Acrylamide, often referred by the acronym AMD, is considered to  
89 be a likely human carcinogen and neurotoxin (EPA US 2010). All PAMs contain some level of residual

90 AMD. However, the amount of residual AMD can vary substantially depending on what measures were  
91 taken during the manufacturing process to maximize the extent of polymerization. Within the EU, all  
92 PAMs are required to contain less than 0.1% (w/w) of residual AMD (European Parliament 1999)  
93 unless they are classified and labeled as a Category 2 carcinogen (European Parliament 2006). More  
94 stringent thresholds are also set depending on additional regulations covering specific uses like, for  
95 example, when PAM-based flocculants are used for the treatment of drinking water (no more than  
96 0.05% of residual AMD). Most of the commercial PAMs products complied with this requirement, even  
97 when they are not marketed for drinking water treatment. Knowledge about the fate of PAM and  
98 associated residual AMD in hydrosystems (transfer, reactivity...) is limited or non-existent in the field  
99 of the use of PAM as a flocculating agent for the treatment of waters. This monomer is water soluble  
100 and unlikely to adsorb to organic and inorganic soil components, potential for leaching and  
101 groundwater contamination are often considered as the major environmental risk associated with the  
102 use of PAMs as flocculants. Several publications have discussed the aquatic toxicity of anionic PAM in  
103 the framework of the struggle against agricultural soil erosion where PAMs are used for soil  
104 stabilization (Barvenik 1994; Seybold 1994; Entry *et al.* 2002; Sojka *et al.* 2000, 2007). These articles  
105 have disregarded its toxicity potential, based largely on theoretical grounds such as the expectation  
106 that toxicity would be mitigated by the complexation with dissolved organic matter (Goodrich *et al.*  
107 1991), because any PAM leaving the field would be rapidly adsorbed to soils (Lentz *et al.* 2002), and  
108 also because the size of the molecule would prevent passage across biological membranes (Stephens  
109 1991). Few authors have also studied the fate of associated residual AMD when PAMs are used for  
110 soil stabilization and demonstrated that in this context AMD undergoes a quick degradation, which  
111 prevents its dispersion in the environment (Labahn *et al.*, 2010; Arrowood, 2008, Shanker *et al.*, 1998;  
112 Lande *et al.*, 1979). Even if these studies are an important source of information, their results and  
113 conclusions cannot be transposed directly to the field of PAM-based flocculants because the  
114 phenomenon that can affect the fate of PAM and associated AMD are slightly different and because  
115 PAMs used for both applications have different properties (molecular weight and charge densities). It  
116 must also be noted that in the field of flocculation, the main vector of dispersion of PAM and AMD in  
117 the environment is water, whether surface or groundwater. In the following parts of this article, the  
118 mechanisms of PAM and AMD transfer and degradation will be thoroughly reviewed and their potential  
119 impacts on the fate of PAM-based flocculants in hydrosystems will be assessed.

120

### 121 **3. Transfer of PAM –based flocculants in hydrosystems**

#### 122 **3.1. Sorption mechanisms**

123 A variety of PAM and AMD molecular and environmental properties interact to affect their transfer in  
124 hydrosystems. These interactions can be significant given the range of PAM use in the environment  
125 and the number of environmental and application factors that can vary. Studies related to the  
126 environmental fate of PAM have been conducted in the framework of several uses of PAMs like soil  
127 stabilization and infiltration enhancement in agriculture (Entry *et al.* 2008; Spofford and Pfeiffer 1996).  
128 Advances in theoretical chemistry have contributed to improvements in PAM performances through  
129 design of molecular conformations optimally suited to given industrial and environmental applications  
130 (Bicerano 1994; Bourani 1998; Bouranis *et al.* 1995; Chamberlain and Cole 1996). Nevertheless,  
131 many of the general principles from these studies are worth noting as a framework for understanding  
132 the environmental behavior of anionic high molecular weight PAMs. Additionally a good deal of  
133 information on the effects of coagulants and flocculants comes from the wastewater treatment  
134 literature, although often from polymers and compounds other than PAM, or in the case of PAM, often  
135 from cationic or nonionic formulations.

136

#### 137 ***Mechanisms of PAM-based flocculants adsorption on mineral particles***

138 The adsorption of PAMs from aqueous media onto mineral surfaces has been largely described in the  
139 literature. For instance the adsorption of PAM on soil and clay mineral surfaces has been  
140 demonstrated to be rapid and irreversible in several studies although the degree of adsorption itself is  
141 dependent on PAM conformation, soil or mineral properties, and soil solutions characteristics  
142 (Hollander *et al.* 1981; Nabzar *et al.* 1984; Nabzar *et al.* 1984; Nabzar *et al.* 1988; Nadler *et al.* 1992;  
143 Pradip and Fuerstna 1980; Tanaka *et al.* 1990; Stutzmann and Siffert 1977; Theng 1982; Tekin *et al.*  
144 2005, Tekin *et al.* 2006; Deng *et al.* 2006; Tekin *et al.* 2010). As PAM adsorption implies multi-  
145 segment interactions, there is a small probability that all train segments are simultaneously detached  
146 from the surface (Theng 1982). Once adsorbed, less than 3% of the adsorbed PAM is removed from  
147 clay minerals after four consecutive washes (Deng *et al.* 2006). Moreover according to Nadler *et al.*  
148 (1992), after adsorption and drying, little desorption of anionic PAM from soil occurs. Michaels and  
149 Morelos (1955) reported that 20% hydrolysis of PAM provided the greatest degree of chain extension,

150 facilitating its adsorption. As PAMs are drawn to mineral particle surfaces, surface-adsorbed water is  
151 driven away because of the stronger attraction for the polymer (Parfitt and Greenland 1970). Sorption  
152 isotherms of PAM generally fit with the linear form of Langmuir equation in comparison with the  
153 Freundlich isotherm (Lu *et al.* 2002; Deng *et al.* 2006; Tekin *et al.* 2005; Tekin *et al.* 2006; Tekin *et al.*  
154 2010). Additionally results of sorption experiments and parameters of Langmuir isotherm found in early  
155 studies are consistent with a favorable adsorption and a high affinity of PAM to soil minerals. This high  
156 affinity is likely due to the mechanisms of multi-segment sorption of the long chain of PAM (Theng  
157 1982).

158 Stutzmann and Siffert (1977) showed that the fixation mechanism of partly hydrolyzed PAM onto clays  
159 in a weakly acidic media is a chemisorption phenomenon. The anionic polyelectrolyte is in fact sorbed  
160 exclusively on the external surface of the clay, where organic molecules undergo protonation and are  
161 retained by ionic interaction. Charge transfer apparently depends on the polarizing power of the  
162 exchangeable cation of the clay and a linear relationship was found between the polarizing power of  
163 the alkali elements and the quantity of material sorbed.

164 Hydrogen bonds are thought as the main mechanism of adsorption of polymers on mineral surfaces.  
165 Adsorption studies of clay-organic complexes have shown that two kinds of H-bonding are possible.  
166 One type of H-bonding can occur between the amide groups of PAMs and the protonated aluminol  
167 groups on the edge of clays (Laird 1997). Depending on pH value, amide group of the PAM can  
168 adsorb either on silanol anchoring sites (at pH 4) or on aluminol anchoring sites (at pH 10) (Pefferkor  
169 1987). Another type of H-bonding was proposed between carbonyl groups and edge aluminols (Laird  
170 1997). Infrared analysis of clay-organic complexes has shown H-bonding between amide groups of  
171 PAM and water molecules in the hydration shell of exchangeable cations of a smectite (Deng *et al.*  
172 2006). Finally, ion-dipole interaction has been proposed in controlling the adsorption of PAM on  
173 smectite. This interaction takes place between exchangeable cations of smectite and the carbonyl  
174 oxygens of amide groups ( $\text{CONH}_2$ ) of PAM (Deng *et al.* 2006).

175 For anionic PAMs, adsorption on kaolinite, illite and quartz is enhanced in the presence of divalent  
176 cations through a cationic bridge process. Cationic bridges take place between divalent cations and  
177 negative groups of anionic polymer on one hand and divalent cations and negatives sites of mineral  
178 surfaces on the other hand. This binding mode occurs at high concentrations of divalent cations ( $\text{Ca}^{2+}$ ,

179  $Mg^{2+}$ ), and enhances the sorption of anionic polymers on negative mineral surfaces (Theng 1982  
180 Chiappa *et al.* 1999).

181 Adsorption of PAMs on the mineral fraction is correlated with size of particles. Clays being the mineral  
182 components with the lowest size have more affinity for PAMs than larger particles like sand and silt (Lu  
183 *et al.* 2002; Gravelieng *et al.* 1997; Malik and Letey 1991). Clays with a high specific outer-surface  
184 area such as montmorillonite and smectite are better PAMs adsorbents than clays with low specific  
185 outer-surface areas such as kaolinite and illite (Lu *et al.* 2002; Deng *et al.* 2006; Inyang and Bae  
186 2005). Additionally the charge repulsions confer to anionic PAM coil an extended conformation making  
187 difficult its entry in the interlayer space of clay minerals (Stuzmann and Siffert 1977). Nevertheless, a  
188 small amount of PAM molecules has been shown to penetrate in the interlayer space of  
189 montmorillonite due to its large interlayer space (Inyang and Bae 2005). It was also shown that  
190 adsorption of PAM increased on montmorillonite when the degree of hydrolysis of PAM increased.  
191 PAM indeed tends to take the conformation of an extended chain when it is highly hydrolyzed.  
192 Therefore the adsorption of PAM depends on the configuration and the volume of the solvated  
193 macromolecule (Stuzmann and Siffert 1977). In contrast, a change of molecular weight has practically  
194 no influence upon the adsorption ratio.

195

### 196 ***Physico-chemical parameters influencing the adsorption of PAM: pH, ionic strength and*** 197 ***temperature***

198

199 The pH value influences both the structure of adsorbent, adsorbate and adsorption mechanism of  
200 PAMs on solid surfaces. The effect of pH on the polymer consists on load changes of functional  
201 groups by the phenomenon of protonation or deprotonation. At a high pH value, the carboxyl group (-  
202 COOH) of polymer can be dissociated as follows: ( $-COOH = -COO^- + H^+$ ), giving the polymer a  
203 negative charge. Furthermore, amides groups can be hydrolyzed and generate carboxyl groups (-  
204  $CONH_2 = -COOH + COO^-$ ) (Deng *et al.* 2006). The net charge of natural solids like clays and silicates  
205 also depends on the pH. For instance the edge sites of aluminosilicates minerals are amphoteric  
206 groups, able to fix or release protons. Thus, the surface charge can be positive when pH is inferior to  
207 PZC (point of zero charge), neutral when pH is equal to PZC, or negative when pH is superior to PZC.  
208 pH values inferior to the PZC induce the protonation of aluminol groups which confers to solid surfaces



209 a positive net charge. Inversely, at pH values superior to the PZC, aluminols sites and silanols sites  
210 are deprotonated, the surface charge becomes negative. Unlike cationic PAMs, the sorption of anionic  
211 PAMs is promoted when the surface is positively charged. This is mainly due to the reduction of  
212 electrostatic repulsions between negatives charges of polymers and the negative charges of the  
213 mineral surface. When  $pK_a[\text{COOH}/\text{COO}^-] < \text{pH} < \text{PZC}$ , attraction of carboxylic groups towards  
214 aluminol groups is enhanced since PAM is negatively charged and the surface is positively charged.  
215 When  $\text{pH} > pK_a[\text{COOH}/\text{COO}^-]$  and  $\text{pH} > \text{PZC}$ , the complete dissociation of carboxylic groups  
216 reinforces the negative charges of PAM and the surface is negatively charged. This results in the  
217 decrease of the adsorption of PAM (Lee *et al.* 1991). The adsorption of PAM tends toward zero when  
218 the pH value still increases because of the very large electrostatic repulsions and the sharp reduction  
219 of AlOH sites (Deng *et al.* 2006; Lee *et al.* 1991).

220

221 The ionic strength is also an important parameter. Benhur *et al.* (1992) found that the adsorption of  
222 PAMs onto illite and montmorillonite clays was generally in the order cationic > nonionic > anionic  
223 regardless of the electrolyte content of the solvating water. Water that was more saline and sodic  
224 reduced the adsorption of cationic and nonionic PAMs but increased adsorption of anionic PAMs. The  
225 results obtained in saline media may be explained by a size decrease of the PAM macromolecules,  
226 allowing a closer approach to the surface of the mineral substrate, and by a chain lengthening as the  
227 degree of hydrolysis increases, induced by the electrostatic repulsions between the  $\text{COO}^-$  groups of  
228 the medium. Aly and Letey (1988) found that the adsorption of anionic PAMs in water of electrical  
229 conductivity (EC)  $0.7 \text{ dS m}^{-1}$  was greater than for water of EC of  $0.05 \text{ dS m}^{-1}$ . Entropy change as water  
230 is displaced is an important actuating force in bringing about adsorption of negative and nonionic  
231 polymers to negatively charged clay surfaces (Lyklema and Fleer 1987; Theng 1982). As the  
232 adsorption of anionic PAMs on mineral surfaces is governed by the competition between polymer  
233 attractive interactions with solid surfaces and repulsive electrostatic forces (Lecourtier *et al.* 1990), the  
234 sorption of anionic polyacrylamide is increased when electrostatic repulsions are screened by cation  
235 bonding on the negatively charged surface sites (Lu *et al.* 2002; Lu and Wu 2003). It has also been  
236 demonstrated that the adsorption of anionic PAM on the edge surfaces of kaolinite is strongly  
237 dependent on salinity (Lee *et al.* 1991). Indeed, two effects have been reported. First, the adsorption  
238 of mono and divalent cations from the electrolyte on the negatively charged surface screens the

239 electrostatic repulsion between anionic PAM and the mineral surface. Secondary, since divalent  
240 cations are involved in the cationic bridge the sorption saturation of PAM is higher in the presence of  
241 divalent cations than in the presence of monovalent cations (Lu *et al.* 2002). Moreover cation  
242 enhancement of sorption is more effective in fine-textured soils than in coarse-textured soils.

243  
244 Finally, studies conducted on the effect of temperature on the adsorption of anionic PAM have shown  
245 contradictory results. Increasing temperature (from 20°C to 60°C) was found to increase the  
246 adsorption of anionic polyacrylamide on kaolinite, sepiolite and perlite (Mpofu *et al.* 2004; Tekin *et al.*  
247 2005; Tekin *et al.* 2006; Tekin *et al.* 2010). This effect was attributed to two phenomena. Firstly,  
248 increasing temperature results in a break of polymer-water hydrogen bonds that promotes the  
249 polymer-polymer interactions. The polymer thus adopts a more compact conformation that will adsorb  
250 more than the expanded one (Mpofu *et al.* 2004). Secondly, increasing temperature may produce a  
251 swelling effect within the internal structure of the clay so the PAM can penetrate further (Tekin *et al.*  
252 2005).

253

## 254 **3.2. PAM Transfer**

### 255 ***Transfer in water***

256 Both molecular properties and product preparation can influence PAMs behavior in hydrosystems  
257 (Callebaut *et al.* 1979). Results from monitoring of PAM concentrations in the drainage water of a  
258 tunnel in Sweden where PAM was used showed that PAMs leaked out, both in connection with the  
259 injection and in connection with after-injection using other grouting agents (Weideborg *et al.* 2001).

260

### 261 ***Flocculation***

262 The general principles of flocculation involving PAMs can be easily drawn from the abundant literature  
263 dedicated to the adsorption of PAMs from aqueous media onto natural mineral surfaces (Greenlan  
264 1963; Bottero and Lartiges 1992; Barvenik 1994; Theng 1979; Theng 1982; Sokja *et al.* 2007; Benhur  
265 *et al.* 1992; Haschke *et al.* 2002; Lu *et al.* 2002; Lurie and Rebhun 1997). In an aqueous solution with  
266 sufficient electrolytes, coulombic and van der Waals forces attract suspended particles to anionic  
267 PAM. These surface attractions stabilize the structure of the aggregates by enhancing particle  
268 cohesion. PAM efficiency as flocculants is not only due to adsorption phenomena. As can be seen on

269 Fig. 2, adsorbed PAM can present three different conformations: “train” conformation, “loop”  
270 conformation or “tail” conformation. Loops and tails can adsorb onto other particles enabling gathering  
271 of several particles in a process known as “polymer bridging”. This phenomenon increases with the  
272 molecular weight of PAMs.

273 The charge density plays also an important role in flocculation process. In anionic PAMs, carboxylic  
274 groups are distributed regularly on the molecular chain, which enhances the polymer extension by  
275 electrostatic repulsion. On another hand, those charges decrease the adsorption onto natural particles  
276 charged negatively (like clay particles). The use of PAMs poorly charged and with a high molecular  
277 weight usually decreases the competition between both phenomena and favor bridging processes.  
278 Small amounts of dissolved  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  can also help by shrinking the electrical double layer  
279 surrounding particles and bridging the anionic surfaces of particles and anionic PAM molecules. The  
280 mechanisms by which flocculation of suspended sediments takes place in the presence of  
281 polyelectrolytes was studied by Ruehrwein and Ward (1952) who associated this effect with  
282 stabilization of soil and resistance to dispersion. Benhur and Letey (1989) and Benhur *et al.* (1989)  
283 attributed this effect to the mechanism that reduced particle dispersion when sprinkler irrigating with  
284 PAM, which in turn reduced surface sealing and slowed the reduction of infiltration rate.

285 At temperatures from 6 to 29 °C, flocculation for a variety of inorganic and polymer compounds tended  
286 to be slower and flocs tend to be smaller than at higher temperatures (Fitzpatrick *et al.* 2004; Hanson  
287 and Cleasby 1990). Furthermore, floc strength seems to vary with the shear conditions of the flow  
288 media in which flocs are formed. Larger flocs formed at higher temperatures are more easily disrupted  
289 and less capable of reformation than flocs formed at lower temperatures (Fitzpatrick *et al.* 2004;  
290 Yeung and Pelton 1996; Yeung *et al.* 1997).

291 PAM solutions usually promote flocculation of suspended solids at low concentrations but can have  
292 the opposite effects at higher concentrations, where the large physical domain of the macromolecules  
293 themselves interfere with flocculation and actually stabilize dispersed suspensions as viscosity  
294 increases. When there is too much of PAM added in the suspension, a compression of the polymer  
295 can also be observed on the particle and there is not enough sites available for bridging, which  
296 decreases the flocculation efficiency. The concentration, at which the effects reverse, depends on  
297 several factors including PAM molecular weight, charge and conformation of the molecule, size and  
298 chemistry of the dispersed solids, and chemistry of the water, particularly its electrical conductivity

299 (Sato and Ruch 1980). The treatment of an effluent containing suspended particles requires  
300 determining precisely the optimal concentration of PAM which must be added in order to perform an  
301 efficient flocculation. This concentration is usually proportional to the particles concentration and is  
302 almost always below 1 mg.L<sup>-1</sup> of suspension.

303

#### 304 ***Transfer with suspended matter***

305 A study measured PAM concentrations in 167-m-long PAM-treated tail ditch that received this runoff  
306 (Lentz *et al.* 2002). The soil was a Portneuf silt loam (coarse-silty, mixed, superactive, mesic  
307 applications Durinodic Xeric Haplocalcid) with a 1.5% slope. PAM was adsorbed to soil and removed  
308 solution as the streams traversed the soil-lined channels. The removal application rate increased with  
309 the stream sediment concentration. Additionally a previous study reported that PAM applications of 1  
310 to 2 mg.L<sup>-1</sup> controlled furrow erosion similarly on 1.5% sloping fields (Lentz and Sojka 2000). The  
311 linear PAM molecule assumes the form of a hydrated random coil when dissolved in water. Solvated  
312 PAM molecules in the furrow stream collide with soil particles when treated water infiltrates into soil or  
313 when turbulent flow drives the molecules against entrained sediment or the wetted soil perimeter. The  
314 dissolved high molecular weight polymers are in fact readily adsorbed to soil particles via electrostatic,  
315 hydrogen, and chemical bonding, and by displacement of inner solvation-sphere water molecules  
316 (LaMer and Healy 1963; Mortland 1970; Jin *et al.* 1987; Malik *et al.* 1991; Laird 1997). As a result,  
317 incoming PAM is bound to soil in the upper 1 to 5 cm of the profile (Malik *et al.* 1991). Dry soil adsorbs  
318 more polymers than wet soils because sorbed water reduces the number of potential soil binding sites  
319 (Chang *et al.* 1991). PAM is adsorbed to particulate matter and flocculates soils suspended in water.  
320 Polymer adsorption on soil occurs rapidly during the first minutes of exposure but may continue at a  
321 reduced rate for several hours or days (Van de Ven 1994). In batch tests (soil, water, and dissolved  
322 PAM mixed in a shaker), Nadler *et al.* (1992) reported that little or no polymer desorbed from the soil  
323 while it remained wet, and the polymer became irreversibly bonded to the soil upon drying. In flowing  
324 systems, Lee and Fuller (1985) found that polymer adsorption rate decreased with increasing velocity  
325 of flow. Polymer desorption did not occur under quiescent conditions but was observed when the  
326 adsorbent material was subjected to flow shear. Desorption increased with the increasing flow velocity.  
327 These authors also concluded that adsorbed PAMs do not penetrate soil aggregates, but only coat  
328 and stabilize their surfaces. Malik and Letey (1991) and Nadler and Letey (1989) used tritium-labeled

329 polymers to determine sorption isotherms of several types of polyanions on Arlington sandy loam  
330 (coarse-loamy, mixed, thermic Haplic Durixeralfs). They interpreted their results as showing that  
331 polymer sorption was restricted to the external surfaces of soil aggregates. A similar conclusion was  
332 reached by ElHardy and Abd ElHardy (1989) who saw only limited intrusion of high molecular weight  
333 PAMs into soil aggregates. Aly and Letey (1988) showed that the water quality of the solvating water  
334 used to apply the polymers also greatly influenced the degree of adsorption.

335 Soils with a high clay or silt content, fine texture and low organic matter content had high sorptive  
336 affinity for PAMs. According to Lu *et al.* (2002), organic matter decreased the amount of anionic PAM  
337 adsorption on soil. The negative effect of organic matter on PAM sorption was attributed to the  
338 reduction of accessible sorption sites by cementing inorganic soil components to form aggregates and  
339 to the enhancement of electrostatic repulsion between PAM and soil surface by its negatively charged  
340 functional groups. The length of the polymer chain and large number of adsorption sites along the  
341 molecule contribute to PAM's adsorption always attached to the adsorption surface, preventing  
342 removal of the molecule (Nadler and Letey 1989). Because of the demonstrated high sorptive ability  
343 and low mobility (Malik and Letey 1991; Malik *et al.* 1991), PAM is generally regarded as incapable of  
344 penetrating soil more than a few centimeters from the soil surface. This, considered in light of the  
345 relatively slow sorption kinetics of the large PAM molecule (Lu *et al.* 2002), has led to recognition that  
346 the depth of PAM penetration depends on PAM properties, application method, and the soil and water  
347 properties present in the application scenario (Lu and Wu 2003). Using anionic PAMs of 10-15 mg  
348 mol<sup>-1</sup>, Lu and Wu (2003) found that PAM penetration depth was about one-eighth to one-half of the  
349 water penetration depth, with a particularly high PAM retention in the top few centimeters of the soil.  
350 The PAM retained in the top 0-2 mm of soil ranged from 16% to 95% of the total applied. PAM  
351 retention was greater at shallow depths when solution contact with the soil was favored by pore  
352 arrangement, contact time, and drier soil conditions on addition of PAM solutions.

353

### 354 **3.3. Transfer of AMD**

355 Mobility of AMD in soils has been little studied. Adsorption of AMD on kaolinite, montmorillonite and  
356 anionic, cationic, and hydrophobic resins is insignificant (Brown *et al.* 1980), at acrylamide  
357 concentrations between 500 µg L<sup>-1</sup> and several mg L<sup>-1</sup>. Arrowood (2008), who studied the adsorption  
358 of acrylamide on different soils (silica sand, coarse sandy soil, loam soil) at AMD concentrations lower

359 than those tested by Brown *et al.* (1980), observed acrylamide adsorption on the three soils with better  
360 affinity for the clay one. The authors concluded that the adsorption of AMD was inversely proportional  
361 to initial concentrations of acrylamide and remains insignificant when compared to the ability of  
362 bacteria to breakdown acrylamide in natural environment. The low affinity of AMD to soil and clay  
363 minerals in particular is likely due to its high hydrosolubility and small size (Sojka *et al.* 2007). Analysis  
364 of a lake water, directly connected to groundwaters, suggest a likely transfer of AMD due to its  
365 infiltration in soils (Junqua *et al.* 2013). The half-life of acrylamide monomer in rivers ranges from  
366 weeks to months (Brown *et al.* 1980).

367

#### 368 **4. Degradation of PAM-based flocculants**

369

##### 370 ***4.1. Mechanisms of PAM degradation***

371 PAM-based flocculants degradation has been mainly studied for PAMs in aqueous solutions. The  
372 degradation of PAMs results in a reduction of the molecular weight of the polymer and can adversely  
373 affect the performance of such aqueous solutions in their commercial application as flocculants.  
374 Degradation involving chain scission is accompanied by a significant change in the rheological  
375 properties of the polymer solutions and this has often been used to monitor and express the extent of  
376 the degradation. A correlation between the reduction in solution viscosity and performance as a  
377 flocculating agent has been demonstrated in number of references. However the factors leading to  
378 such degradation and the mechanisms of the degradation processes are not completely elucidated.

379 In environmental applications (such as in flocculation processes), very few experiments have been  
380 conducted to quantify the PAM degradation in natural hydrosystems. Some reports have indicated that  
381 polyacrylamide, in the presence of sunlight and glyphosate, photolytically degrades to acrylamide  
382 monomer and this is a direct introduction of acrylamide into agricultural areas (Smith and Oehme  
383 1993, Smith *et al.* 1996; Smith *et al.* 1997). The main difficulty encountered is due to the lack of simple  
384 methods to analyze and quantify PAM in this context. Thus what is known about PAM degradation is  
385 drawn from sparse reports, often using smaller molecular weight PAMs, or from indirect measurement  
386 of decomposition. Most studies report slow degradation rates as a result of mechanical, photochemical  
387 and biological processes.

388

389 ***Mechanical degradation***

390 Aqueous solutions of PAM or floc suspensions can both undergo mechanical degradation due to a  
391 variety of processes such as stirring, pumping, injection and movement through porous media and  
392 direct mechanical load. In the aggregate treatment plant, mechanical degradation of the PAM can  
393 occur due to a shearing hydrodynamic field (mixing of the PAM-based flocculants with the process  
394 waters and transportation of the sludge to tailings ponds along pipelines through pumping) or by direct  
395 mechanical load (solid/liquid separation and sludge dewatering with a press-filter).

396 It was reported that a shearing hydrodynamic field induced chain bond scissions leading to a decrease  
397 in the molecular weight of the polyacrylamide and then to a decrease in intrinsic viscosity of PAM  
398 solutions (Paterson and Abernathy 1970; Abdel-Alim and Hamielec 1973; Nagashiro *et al.* 1975;  
399 Povkh and Chernyuk 1986; Nguyen and Boger 1998; Avadiar *et al.* 2013). In particular, mechanical  
400 degradation of polymers used for drag reduction in a turbulently flowing liquid has been studied by  
401 several authors (Rho *et al.* 1996; Vlachogiannis *et al.* 2003; Pereira and Soares 2012). Losses in the  
402 effectiveness of the polymers in reducing drag under flow were attributed either to fractures of the  
403 PAM molecules leading to changes in the molecular weight distribution or to the fact that some or all of  
404 the degradation could be associated with a decrease of aggregation in a shear flow (Wade and Kumar  
405 1972; Vlachogiannis *et al.* 2003).

406 Regarding mechanical degradation of flocs, Tang *et al.* (2001) showed that the mechanical strength of  
407 the floc depends on both the interparticle forces and on the floc density. In general, the rupture of floc  
408 is classified either as “surface erosion” or as “large-scale fragmentation” (Yeung and Pelton 1996).  
409 Erosion is the separation of small particles from the floc surface and it is attributed to shearing  
410 stresses on the floc surface. Floc breakage during shear is suspected to involve the rupture of  
411 hydrogen bonds (Senden *et al.* 2000; Taylor *et al.* 2002). Fragmentation refers to the break-up of flocs  
412 into pieces of comparable size caused by pressure gradients across the entire body. Since flocs  
413 break-up occurs at the weakest point in the floc structure, this usually results in more compact  
414 aggregates, though smaller than the initial flocs. According to Caulfield (2002), it cannot be stated that  
415 under the influence of a mechanical load polyacrylamides degrade to form their constituent monomeric  
416 units such as acrylamide. Moreover, several authors reported that the flocs formed using  
417 polyelectrolytes such as PAM are reformed after being broken up but do not regain their original size  
418 and structure and form a new structure more compacted (Nguyen and Boger 1985; Leong *et al.* 1987;

419 Spicer *et al.* 1998). An example of experiments on mechanical degradation was performed by Rasteiro  
420 (2008) who studied the degradation under hydrodynamic shearing induced by a recirculating pump of  
421 two high molecular weight cationic PAMs used for the flocculation of precipitated calcium carbonate.  
422 For the pump speeds tested, the flocs size decrease was attributed to floc erosion but it was also  
423 stated that flocs reach another equilibrium state where aggregation and fragmentation rates are the  
424 same. Moreover, it was reported that erosion may not depend on the flocculant concentration since it  
425 depends on the interparticles bonds of the flocs; however erosion increased with the pump speed.  
426 Reflocculation occurred only for the higher flocculant concentrations due to the excess of polymer and  
427 most of the flocs resulting from the reflocculation process were observed to be more compact than the  
428 original ones Rasteiro (2008).

429 Mechanical load can also induce enough shear-induced stress to cause bond scission and the  
430 formation of a macromolecular radical (Caulfield 2002). For example, it was observed that filtration  
431 under pressure of thickened suspensions from PAM flocculation of bauxite residue or brown coal lead  
432 to significant breakdown in network structures and reduction in yield stresses (Nguyen and Boger  
433 1985; Leong *et al.* 1987). The implementation of shear stresses that are higher than the initial yield  
434 stresses of flocs suspension leads then to breakdown the intricacy involved within these complex  
435 network structures (Avadiar *et al.* 2013).

436 Therefore, mechanical degradation of aqueous solutions of PAM or floc suspensions often involves  
437 irreversible changes of the polymeric material including a marked reduction in viscosity and a scission  
438 of the main chain bond and thus the generation of free radicals. However, none of the studies found in  
439 the literature suggest that PAMs degrade to AMD under mechanical constraints.

440

#### 441 ***Photodegradation of polyacrylamide***

442 PAM photodegradation is reported to be a free radical process that can lead to a cleavage of the  
443 polymer backbone (bond scission) and the formation of lower molecular weight products (Decker  
444 1989; Caulfield *et al.* 2002). The C-C, C-H and C-NH bonds in PAMs have bond strengths of 340, 420  
445 and 414 kJ mol<sup>-1</sup> which can be cleaved by wavelengths of 325, 288 and 250 nm respectively (Crosby  
446 1976). Even if the atmosphere absorbs most of the lower spectrum (less than approximately 300 nm),  
447 it still leaves components from the UV and visible regions with sufficient energy to disrupt chemical  
448 bonds (Rabek 1996). The free radicals formed from bond scission are extremely unstable and will



449 react with any suitable substrate. The reactions include combination to reform the saturated carbon-  
450 carbon polymer backbone, disproportionation to form an unsaturated and saturated polymer chain end  
451 and reaction with other entities present. The presence of oxygen in the system has a strong impact on  
452 photodegradation of PAM as well as the impurities contained in the initial polymer.

453 Some authors have studied PAM degradation via advanced oxidation processes, such as Fenton  
454 (Kurenkov *et al.* 2002), H<sub>2</sub>O<sub>2</sub>/UV (Caulfield *et al.* 2002), photo-Fenton (Giroto *et al.* 2008) and  
455 O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>/UV (Ren *et al.* 2006), but these reactions cannot occur in environmental conditions.

456 Most of the studies, dedicated to PAM photodegradation in environmental conditions, focus on the  
457 potential release of AMD. This phenomenon was firstly observed by Smith *et al.* (1996, 1997), in  
458 controlled laboratory conditions and under environmental conditions using an aqueous solution of a  
459 PAM thickening agent (PAM mixed with glyphosate). However these results, their interpretation and  
460 the overall experimental design raised some controversy, particularly the way that PAM was prepared  
461 which could lead to incomplete dissolution of PAM (Kay-Schoemake *et al.* 1998a; Ver Vers 1999;  
462 Caulfield *et al.* 2002). Ver Vers (1999) reported work using analogous degradation conditions but  
463 yielding different results. This study points out problems in proper dilution of the emulsion and  
464 attributes the detection of small amount of AMD over time to the gradual release of unreacted AMD  
465 contaminants from the incompletely dissolved emulsion. The author concluded that PAM does not  
466 degrade to AMD monomer in the presence of sunlight, even in presence of glyphosate. Kay-  
467 Shoemake *et al.* (1998b) showed that the treatment of solutions of high molecular weight anionic  
468 PAMs with UV radiations reduced the PAM chain length. They found also that the photodegraded  
469 PAM samples did not support bacterial growth as sole source of carbon. As it is well recognized that  
470 acrylamide is completely biodegradable (see section VI), these results suggest that AMD is not formed  
471 during the photodegradation of the polyacrylamide. El-Mamouni *et al.* (2002) investigated the  
472 photodegradation of a PAM solution (PAMs molecular weight ranging between 10<sup>5</sup> and 10<sup>6</sup> daltons)  
473 exposed to UV irradiation (254 nm) prior to biomineralization. They observed a shift in the molecular  
474 weight distribution, which demonstrates the degradation of the PAM chains into oligomers. A study  
475 leaded by Caulfield *et al.* (2003) has reported that strong UV radiation at 254 nm released AMD from  
476 solutions of a nonionic PAM. However, the release was very small. They also noted a reduction in  
477 viscosity of the polymer solution, attributed to a lowering of the molecular weight consistent with  
478 polymer chain scission. Suzuki *et al.* (1978, 1979) also reported a number of low molecular weight

479 PAM decomposition products when degraded using strong UV irradiation in the presence of ozone but  
480 didn't notice any AMD monomer release. It must be noted that the studies of Kay-Schoemake *et al.*  
481 (1998a), El Mamouni *et al.* (2002), Caulfield *et al.* (2003) and Suzuki *et al.* (1978, 1979) used UV  
482 irradiations which are much more energetic than the sunlight's wavelengths. These studies cannot  
483 confirm the attenuation of PAM by sunlight in natural environments.

484 Holliman *et al.* (2005) submitted samples of field-conditioned cross-linked PAM gels to a 365-nm UV  
485 radiation in a daily light-dark cycle during 5 months without detectable acrylamide release. On the  
486 other hand, they observed a gradual loss of gel properties they attribute to polymer chain scission  
487 resulting in the production of PAM fragments. More recently, a study by Woodrow *et al.* (2008)  
488 reported AMD release resulting from sunlight irradiation of aqueous polyacrylamide/iron mixtures. A  
489 solution of commercial anionic PAM with different concentration of  $\text{Fe}^{3+}$  (from 0.02 to 4.4 ppm) was  
490 submitted to UV irradiation in a simulator able to closely approximate solar irradiance in the  
491 troposphere (equivalent to late summer noontime sunlight at 40° latitude – 300nm). Without  $\text{Fe}^{3+}$ , only  
492 polymer chain scission was observed through HPLC measurement and decline of average molecular  
493 weight. In the presence of  $\text{Fe}^{3+}$ , acrylamide release was also observed at acid/neutral pH whereas at  
494 alkaline pH (~ 8.0), PAM/ $\text{Fe}^{3+}$  remained stable under irradiation.

495 In conclusion, PAMs are susceptible to a wide range of photodegradative processes depending on the  
496 reaction conditions. Photodegradation results in irreversible changes in both physical and chemical  
497 properties of the PAMs, but the release of acrylamide still remains in question and would require  
498 further clarification. Moreover photodegradation of PAM was not directly demonstrated in  
499 environmental conditions in hydrosystems with field studies but only in laboratory aqueous solutions  
500 without the presence of mineral particles.

501

### 502 ***Biological degradation***

503 Whether they are used as flocculants for water clarification or in agricultural applications for the control  
504 of soil erosion as well as in petrochemical productions to enhanced oil recovery, PAMs are in contact  
505 with micro-organisms and can be subject to biodegradation. Most of the studies on the degradation of  
506 PAM in agricultural soils concluded that the biodegradation of PAM did not result in the formation of  
507 monomer (AMD) and that PAMs were generally resistant to microbial degradation (Caulfield *et al.*

508 2002). During these applications, the degradation seemed to be most likely due to environmental  
509 factors such as UV light or chemical degradation (Seybold 1994).

510 The ability of bacteria to utilize PAM as sole nitrogen or sole carbon sources has been examined. Kay-  
511 Shoemake *et al.* (1998a) demonstrated that PAM can be used as an organic nitrogen source for  
512 microorganisms in soil. A bacterial growth was observed when cultures were produced on soil  
513 samples with no other source of nitrogen. Intracellular and extracellular amidase activities were  
514 detected in all the enrichment cultures able to use PAM as sole source of nitrogen and in soils treated  
515 with PAM. Haveroen *et al.* (2005) also demonstrated that the addition of PAM stimulates microbial  
516 activities and methanogenesis in anaerobic environments that are poor in nitrogen sources. In another  
517 study, Kay-Shoemake *et al.* (1998b) show that indigenous bacteria, commonly found in soils, were  
518 enabled to use PAM as sole carbon source. These results suggest that these microorganisms could  
519 hydrolyse the amine group but were unable of cleaving the main carbon chain of the polymer.

520 PAMs are high molecular weight polymers and are unable to pass biological membranes, so the  
521 biodegradation of these polymers requires the action of extracellular enzymes, like amidases, to  
522 degrade the carbon chain. These characteristics limit the potential of biodegradation and can be at the  
523 origin of the partial degradation of PAM (Caulfield *et al.* 2002). Few studies demonstrated the  
524 biodegradation of these compounds. Nakamiya and Kinoshita (1995) were the first ones to report the  
525 isolation of two PAM-degrading bacterial strains from soil samples. These strains, identified as  
526 *Enterobacter agglomerans* A and *Azomonas macrocytogenes* B, were able to reduce the molecular  
527 weight of a synthesized PAM and to degrade about 15 to 20% of the initial concentration of PAM in a  
528 synthetic medium. Another study (Matsuoka *et al.* 2002) reported the isolation of two PAM-degrading  
529 bacteria strains from soil, identified as *Bacillus sphaericus* n°2 and *Acinetobacter sp.* No. 11. These  
530 strains grew on medium containing PAM as sole carbon and nitrogen sources and reduced,  
531 respectively, by 16% and 19% of the initial PAM concentration. However, the degradation of PAM did  
532 not result in the formation of AMD. As previously indicated, the biodegradation of PAM require  
533 extracellular amidase activities. These enzymes seem to be enabled to liberate AMD directly from  
534 PAM but can produce lower molecular weight polymers and can alter the nature of the substituents  
535 attached to the polymer backbone (Caulfield *et al.* 2002, Kay-Shoemake *et al.* 1998b).

536 More recently, several bacterial strains isolated during oil recovery process have demonstrated the  
537 ability to degrade Hydrolysed Polyacrylamide (HPAM) or PAM (Ma *et al.* 2010; Wen *et al.* 2010; Bao *et*

538 *al.* 2010). Ma *et al.* (2010) have isolated a *Clostridium bifermentans* H1 strain which can use HPMA as  
539 sole carbon source and can hydrolyse the amine to carboxyl group by the molecule chain amine  
540 hydrolysis. In their experimental conditions, the removing efficiency for HPAM reached 30.8 %. Wen *et*  
541 *al.* (2010) reported the isolation of two strains of *Bacillus cereus* HWBI and *Bacillus flexu* HWBII from  
542 activated sludge and oil-contaminated soil. Both strains were able to grow on a medium containing  
543 PAM as sole carbon and nitrogen source and to degrade PAM at different rates. After 72 hours of  
544 cultivation in a mineral salt medium (MSM) supplemented with 60 mg.L<sup>-1</sup> of PAM at 30°C, more than  
545 70 % of the PAM was consumed and PAM was cleaved into smaller molecules. Another study  
546 reported the isolation of two *Bacillus sp.* strains, named PM-2 and PM-3 from production water after  
547 polymer flooding in an oilfield (Bao *et al.* 2010). The PAM removal efficiencies observed were lower  
548 than others studies. After 7 days of cultivation at 40°C in a basal medium containing 0.5 g.L<sup>-1</sup> of HPMA  
549 as a sole source of nitrogen, 36.3 % of the HPMA was degraded. Lower HPMA removal efficiencies  
550 were observed when HPMA was used as sole carbon and nitrogen sources. These results suggested  
551 that the PAM-degrading strains obtained more nutriments from the amide sides groups of HPAM using  
552 amidase activities compared to cleaving the main carbon chain backbone (Kay-Shoemake *et al.*  
553 1998b).

554 Although the biodegradation of PAM has been studied with isolated strains in pure culture, in practical  
555 work, the biodegradation of PAM requires interactions between several microbial species. Recently,  
556 Liu *et al.* (2012) have described a PAM-degrading granular sludge which degraded PAM. The PAM-  
557 degrading granules which were mostly composed by filamentous and bacilli bacteria used PAM as  
558 sole carbon and nitrogen source and degrade PAM at a high rate. PAM concentration was decreased  
559 from 66.7 mg.L<sup>-1</sup> to 32.9 mg.L<sup>-1</sup> in 24 hours but no AMD monomer was detected during the  
560 biodegradation process. The main intermediate product was identified as polyacrylic acid with a low  
561 molecular weight.

562 These studies presented in this review demonstrated that PAM can be subjected to microbial  
563 degradation. PAM was principally used as a nitrogen source by hydrolysis of the amine groups from  
564 the polymer. The presence of glucose at low concentrations which can be used as co-metabolism  
565 substrate can improve the PAM degradation but the biodegradation efficiency seems to be affected by  
566 the initial concentration of PAM (Wen *et al.* 2010). The biodegradation of PAM induced changes in the  
567 structure of the polymer (reduction of the molecular weight) but no evidence of release of AMD

568 monomer has been found. Although amidases are involved in the mechanism of degradation of PAM,  
569 the reaction pathway for the microbial degradation of PAM is not clearly identified.

570

## 571 **4.2. Mechanisms of acrylamide degradation**

### 572 ***Bio-degradation of acrylamide***

573 AMD has an inhibitory effect on sulfhydryl proteins and hence on growth of microorganisms (Cavins  
574 and Friedman 1968). However, some microorganisms are able to grow in the presence of AMD and  
575 degrade this molecule. The microbial degradation of AMD has been demonstrated for several decades  
576 in studies investigating the behavior, fate and impacts of AMD in natural environments amended with  
577 PAMs. Microbial degradation was shown to occur in soils (Shanker *et al.* 1990, Shukor *et al.* 2009a),  
578 sediments (Labahn *et al.* 2010), wastewater (Buranasilp and Charoenpanich 2011, Nawaz *et al.* 1996)  
579 and hydrosystems (Labahn *et al.* 2010) and is mediated by microorganisms commonly presents in  
580 these environments. Most studies deal with the biodegradation of AMD by bacteria but some fungi  
581 have also been shown to degrade AMD. In particular, filamentous fungi such as some strains of  
582 *Aspergillus oryzae* have interesting AMD degradation ability (Wakaizumi *et al.* 2009).

583 Among bacteria having an AMD degrading ability, most documented belong to species within the  
584 genera *Bacillus* (Shukor *et al.*, 2009a), *Pseudomonas* (Labahn *et al.* 2010, Sathesh Prabu and  
585 Thatheyus 2007, Shanker *et al.* 1990, Shukor *et al.* 2009b) and *Rhodococcus* (Nawaz *et al.* 1998).  
586 However it is now establish that this activity is more widespread among bacteria such as *Enterobacter*  
587 *aerogenes* (Buranasilp and Charoenpanich 2011), *Enterococcus faecalis* (Buranasilp and  
588 Charoenpanich 2011) *Klebsiella pneumoniae* (Nawaz *et al.* 1996), Burkholderia sp. , *Xanthomonas*  
589 *maltophilia* (Nawaz *et al.* 1993), *Kluyvera georgiana* (Thanyacharoen *et al.* 2012), *Variovorax*  
590 *boronicumulans* (Liu ZH. *et al.* 2013), *Geobacillus thermoglucosidasius* (Cha and Chambliss 2013),  
591 *Ralstonia eutropha* (Cha and Chambliss 2011) or *Arthrobacter* strains (Yamada *et al.* 1979). The  
592 concentration of AMD that can be tolerated and degraded depends on the bacterial strain. As an  
593 example and among strains that tolerate the higher concentration of AMD, *Bacillus cereus* is able to  
594 degrade and assimilate AMD at concentrations as high as 3 g. L<sup>-1</sup> (Shukor *et al.* 2009a). Shanker *et*  
595 *al.* (1990) isolated a *Pseudomonas sp.* strain able to degrade 4 g. L<sup>-1</sup> AMD, and *Enterobacter*  
596 *aerogenes* was shown to grew well in the presence of 5 g. L<sup>-1</sup> AMD (Buranasilp and Charoenpanich  
597 2011). It has also been demonstrated that bacteria able to degrade AMD are also able to degrade

598 other aliphatic amides such as acetamide, urea, propionamide (Buranasilp and Charoenpanich 2011;  
599 Shukor *et al.* 2009a).

600 The biodegradation of AMD was first shown to be aerobic but recent studies have demonstrated the  
601 involvement of some bacteria in the anaerobic biodegradation of AMD. In particular, nitrate reducers  
602 have been shown to significantly remove AMD (Labahn *et al.* 2010). These authors also demonstrated  
603 the potential of sulfate- and iron-reducers to degrade AMD. The biodegradation of AMD leads to the  
604 production of acrylic acid (AA) and ammonia (NH<sub>3</sub>). This could explain that AMD degrading  
605 microorganisms use AMD as nitrogen source and sometimes also as carbon source, and that some  
606 bacteria can grow as AMD as the sole sources of N and C (Shukor *et al.* 2009a, Nawaz *et al.* 1994,  
607 1996). From an experimental point of view, AMD can be analyzed directly either by HPLC or by UPLC  
608 depending on the concentration (Togola *et al.*, 2014; Guezennec *et al.*, 2014; Touze *et al.*, 2014;  
609 VerVers, 1999). The degradation of AMD can also be easily monitored by the quantification of  
610 ammonia that can be achieved using indicator such as Phenol red that changes in color (from red to  
611 pink) in the presence of ammonia (Santoshkumar *et al.* 2010).

612 Many studies also underlined that the AMD biodegradation activity of free cells and immobilized cells  
613 can be different. It is in fact usually admitted that immobilized bacterial technology offers several  
614 advantages over the application of free cells in the degradations of pollutants (Nawaz *et al.* 1998):  
615 immobilized cells are known to retain high catabolic activity, can be reused, efficiently mineralize  
616 substrates and offers physical protection from predators and parasites. Nawaz *et al.* (1998) thus  
617 demonstrated that immobilized cells of *Rhodococcus sp.* rapidly degraded 64 and 128 mM AMD in 3  
618 and 5 hours respectively whereas free cells took more than 24 h to degrade 64 mM AMD. Moreover  
619 an AMD concentration of 128 mM inhibited the growth of the free cells. Sathesh Prabu and Thatheyus  
620 (2007) observed that free *Pseudomonas aeruginosa* cells began the degradative activity of AMD only  
621 after incubation of 24 h, while immobilized cells started before 24 h. On the opposite, Buranasilp and  
622 Charoenpanich (2011) showed that AMD degradation by immobilized cells of *Enterobacter aerogenes*  
623 was slower than that of free cells. This could be explained by an inappropriate immobilization process  
624 or difficulties in substrate-cell contact.

625 In addition to the state (free or immobilized) of cells, the biodegradation of AMD is influenced by  
626 several physical factors such as AMD concentration, temperature, pH, the presence of metals. Nawaz  
627 *et al.* (1998) demonstrated that the biodegradation of *Rhodococcus sp.* was lower at low temperature

628 (less than 60% of AMD was degraded at 10°C in 4 h whereas 100 % of AMD was degraded in less  
629 than 3 h at 28°C and 45°C). An incubation temperature of 55°C completely inhibited the degradation of  
630 AMD by this strain. pH 7 favored maximum degradation of AMD, and activity was slower at pH 8 and  
631 very little degradation was measured at pH 6. These authors also demonstrated that nickel and cobalt  
632 slowed the AMD degradation ability (when used at 5 mM) or inhibit (when used at 10 mM) the AMD  
633 degradation ability. The highest rate of degradation of AMD was observed in the presence of iron but  
634 iron concentrations higher than 10 mM inhibited AMD degradation. Sathesh Prabu and Thatheyus  
635 (2007) showed that mercury and chromium inhibited AMD degradation by *Pseudomonas aeruginosa*  
636 while nickel enhanced the process.

637

### 638 ***AMD biodegradation in environmental conditions***

639 If numerous authors have investigated the mechanisms of AMD biodegradation and the  
640 microorganisms involved, very few have quantified AMD biodegradation rate and kinetics in  
641 environmental conditions (temperature, AMD concentration, natural samples...). Those study exhibit  
642 contrasting results (see Table 1) and are difficult to compare since most of biodegradation tests have  
643 been conducted in various range of AMD concentration. The work carried out by Labahn *et al.* (2010)  
644 on natural water and slurry samples spiked with 50 µg.L<sup>-1</sup> AMD show the impact of the temperature on  
645 AMD biodegradation: at 15°C, no degradation is detected after 72h whereas at 25°C, AMD  
646 degradation is complete after 24h. These results confirm those obtained by Nawaz *et al.* (1998) in  
647 controlled laboratory biodegradation study using pure strain of *Rhodococcus* sp (see section above).  
648 Croll (1974) investigated AMD biodegradation in river water samples spiked with 10 µg.L<sup>-1</sup> AMD. One  
649 of the water samples was inoculated with a culture capable of degrading acrylamide. In this case,  
650 AMD degradation was complete after 50h, the lag period being 5h. In water samples which were not  
651 inoculated, the time required to fully degrade AMD reached more than 300h with a lag period of 50h.  
652 Brown (1979) used also river water samples but with higher concentration of AMD (5 mg.L<sup>-1</sup>) and  
653 investigated the influence of light on the biodegradation. In both cases (with and without light), the  
654 results were the same: AMD was fully degraded after 125h with a lag time of 75h. In a more recent  
655 study Ver Vers (1999) demonstrated that the time required for complete AMD biodegradation could  
656 vary from 480h to more than 1100h according to the type of water samples (tap, river and lake). For

657 the author, those differences could indicate that some species in the water may be interacting with  
658 AMD, either accelerating or hindering monomer degradation.

659 The results obtained by these author show that AMD biodegradation is probably influenced by  
660 different types of factors such as the initial AMD concentration, the nature of the media in which the  
661 degradation occurs, its microbial composition... Given the conditions used, the degradation can be  
662 quick but the time required for a complete degradation of AMD is never lower than 1 day. In some  
663 cases several days are needed to degrade the monomer.

664

#### 665 ***Metabolism of acrylamide and enzymes involved in bio-degradation***

666 As mentioned before, AMD can be degraded by microorganisms under aerobic and anaerobic  
667 condition, and serves as nitrogen and/or carbon sources. Despite the fact that microbial degradation of  
668 acrylamide has been explored extensively, the metabolic pathways of degradation are unclear. The  
669 microbial degradation of AMD is initially due to the presence of an enzyme named acylamide  
670 amidohydrolase (E.C. 3.5.1.4) or amidase. Amidases catalyze the hydrolysis of an amide to free  
671 carboxylic acids and free ammonium. In the case of acrylamide, the AMD is deaminated to ammonia  
672 and acrylic acid (AA). These enzymes belong to the family of hydrolases which acts on carbon-  
673 nitrogen bonds other than peptide bonds. They are ubiquitous enzymes that can have various  
674 biological functions and the substrate preferences differ. In their review, Sharma *et al.* (2009) widely  
675 described these enzymes, their uses in industries (and their potential applications in the production of  
676 various chemicals), their structural and biochemical properties, substrates and some purification  
677 protocols.

678 Amidase activity has been identified in various bacteria genus, commonly found in soils, including  
679 *Rhodococcus*, *Bacillus*, *Brevibacterium*, *Alcaligenes* and *Pseudomonas* and in fungi (Kay-Shoemake  
680 *et al.* 1998b, Wakaizumi *et al.* 2009). These micro-organisms are able to produce a single amidase or  
681 numerous isoenzymes during growth with amide substrate. Furthermore, amidases exhibited  
682 enzymatic activity with relatively limited substrate specificity while others are able to hydrolysis a broad  
683 range of spectrum. Among amidases, some have been demonstrated to be involved in the first step of  
684 the AMD-degrading microbial pathways for the catalytic hydrolysis of AMD to acrylic acid and  
685 ammonia (Sharma *et al.* 2009).



686 Although some amidases are constitutive (Nawaz *et al.* 1994), microbial amidases are generally  
687 inducible. It has been demonstrated that amides and AMD can be an inducer of amidase expression  
688 and some carbon sources as glucose or succinate can induce a catabolic repression (Potts and Clarke  
689 1976, Sharma *et al.* 2009). The regulation of amidase (encoded by *amiE* gene) expression has been  
690 study in *Pseudomonas aeruginosa*. In this microorganism, the induction of amidase synthesis is  
691 positively regulated by a regulatory protein encoded by *amiR* and a second regulatory gene, *amiC*,  
692 which negatively regulates amidase expression (Clarke *et al.* 1981, Cousens *et al.* 1987, Wilson. and  
693 Drew 1991, Wilson *et al.* 1993, Wilson and Drew 1995). However, under non-inducing condition, low  
694 levels of amidase activity are always present within the cells. But, the cellular mechanism for induction  
695 or sensing remains unclear because the PAM-polymer is too large to enter the cells and to act as a  
696 direct inducer. Shanker *et al.* (1990) have demonstrated that addition of glutamate or ammonium  
697 sulfate as additional nitrogen source induced increase in degradation potential.

698 Several amidases using AMD as substrate have been purified from AMD-degrading bacteria (Cha and  
699 Chambliss 2013; Egorova *et al.* 2004; Lavrov *et al.* 2010; Liu *et al.* 2013; Makhongela *et al.* 2007;  
700 Nawaz *et al.* 1994; Nawaz *et al.* 1996; Ohtaki *et al.* 2010; Syed *et al.* 2012). The amidases purified  
701 from AMD-degrading bacteria are mainly monomer but that from *Burkholderia sp.* is a homotetramer  
702 (Syed *et al.* 2012). The molecular weight, pH and temperature optima depend on the enzyme (see  
703 Table 2). It has been also demonstrated that the catalytic hydrolysis of AMD by amidase can be  
704 inhibited or enhanced by metals (Nawaz *et al.* 1994). These authors showed that amidase activity of  
705 *Rhodococcus sp.* was enhanced by  $Fe^{2+}$ ,  $Ba^{2+}$  and  $Cr^{2+}$  but not dependent on these cations. On the  
706 other side, the enzyme activity was partially inhibited by  $Mg^{2+}$  and totally inhibited in the presence of  
707  $Ni^{2+}$ ,  $Hg^{2+}$ ,  $Cu^{2+}$  and  $Co^{2+}$ .

708 The biodegradation of AMD requires not only amidase but also enzymes able to metabolize the  
709 intermediate products. Indeed, the biodegradation of AMD induces the production of ammonia and  
710 acrylic acid. However, the subsequent fate of these products is not clear but probably involves specific  
711 catabolic pathways. For AMD degrading-bacteria, AA is probably reduced to generate energy for  
712 bacterial growth but the catabolic pathway is not clear. Wampler and Ensign (2005) suggested that AA  
713 is degraded to  $\beta$ -hydropropionate via hydroxylation reaction, then oxidized to  $CO_2$  or reduced to  
714 propionic acid. Recently, Charoenpanich and Tani (2014) have studied the response of an acrylamide-  
715 degrader *Enterococcus aerogenes* to AMD using proteome analysis. These authors have identified

716 fifteen proteins differentially expressed after growth in presence of AMD and have proposed a model  
717 of metabolic pathway of AMD degradation based on these results (see Fig. 3).

718 Finally, the identification of a “universal” pathway and the enzymes involved in AMD metabolism in  
719 prokaryotes remains a challenge for the optimization of the bioremediation of AMD.

720

## 721 **5. Conclusion**

722 This review summarizes the literature which gives information and scientific data on the fate of PAM-  
723 based flocculants in hydrosystems. PAM and associated AMD have been considered and three main  
724 families of phenomenon have been investigated: transport, adsorption and degradation. Most of the  
725 information gathered in this review was derived from studies conducted in the frame of PAM use for  
726 agricultural application. Due to PAM ability to adsorb onto mineral particles, its transport in surface  
727 water, groundwater and soils is rather limited and restricted to specific conditions. PAM adsorption is  
728 rapid and irreversible, and desorption occurs only at a very small extent. In the contrary, the  
729 adsorption of acrylamide onto particles is very low, which is likely due to its high hydrosolubility and  
730 small size. This low affinity to soil and clay minerals could favor its transfer in surface waters and  
731 ground water, but very few studies reports AMD analysis in natural water. Moreover, several studies  
732 have demonstrated microbial degradation of acrylamide in environments amended with  
733 polyacrylamide. The biodegradation of acrylamide could thus limit its transfer in the environment.  
734 PAMs are high molecular weight polymers and are unable to pass biological membranes, so the  
735 biodegradation of these polymers requires the action of extracellular enzymes, like amidases, to  
736 degrade the carbon chain. These characteristics limit the potential of PAM biodegradation. PAM can  
737 be also subject of photodegradation and mechanical degradation. In any case, most of the studies  
738 report slow degradation rates without AMD release. The main conclusion of this review is that, despite  
739 the abundant literature dedicated to PAM use as flocculants, there is an obvious lack of pertinent and  
740 detailed data about the behavior, the fate and the impact of PAM-based flocculants in environments.  
741 Lot of work has still to be carried out to elucidate the mechanisms of degradation processes as well as  
742 to quantify the transfer of PAM and AMD in hydrosystems.

743

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746

747 **References**

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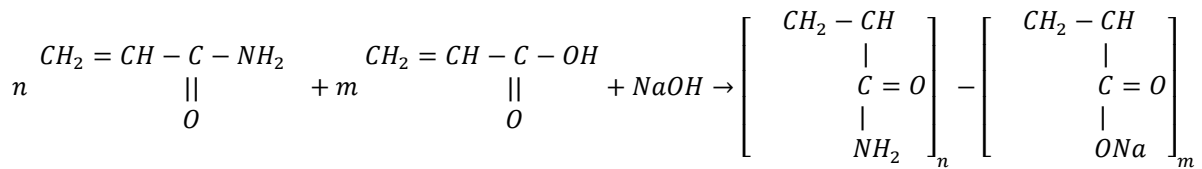
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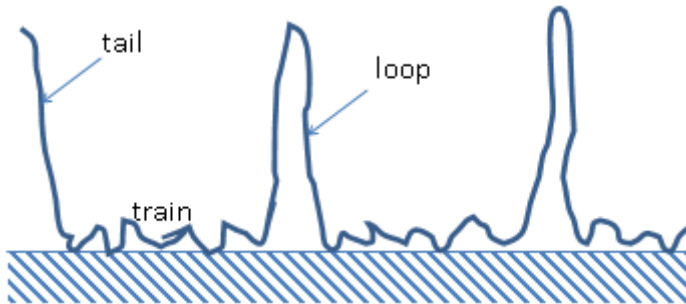
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1132 **Fig. 1 Synthesis pathway of anionic PAMs**

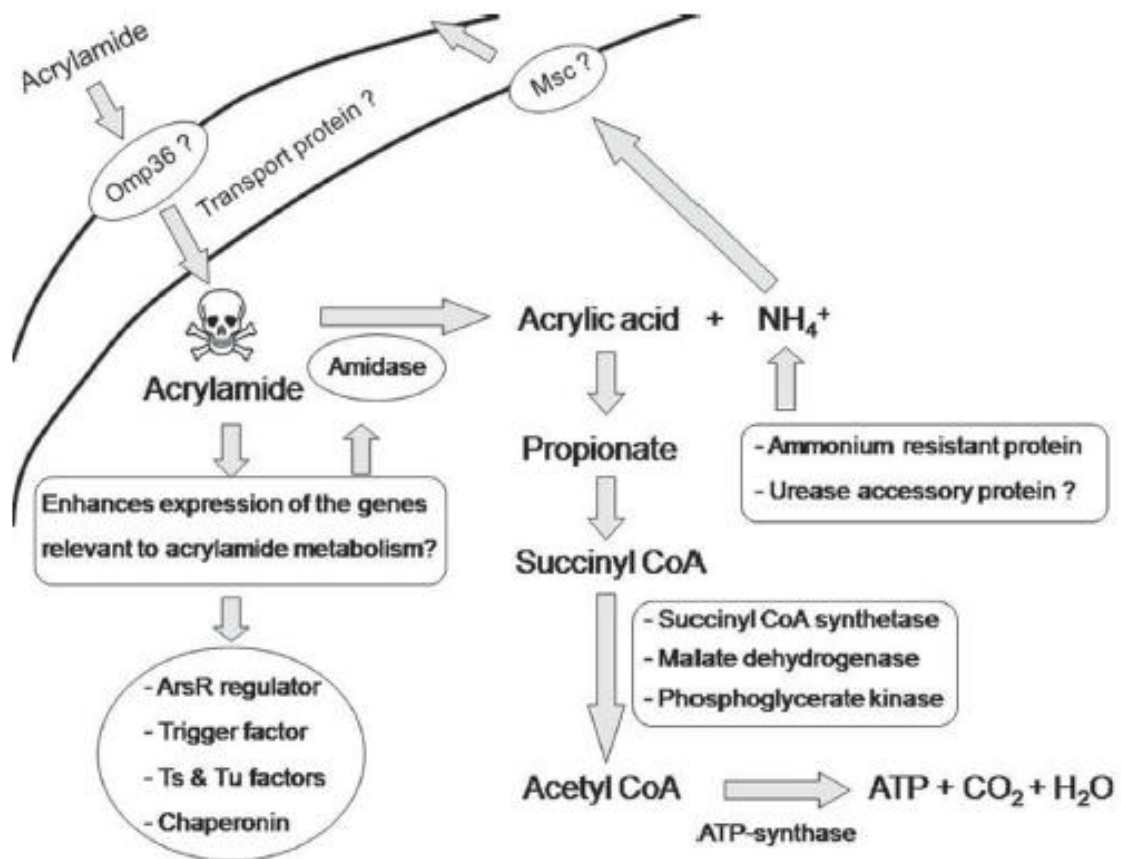
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1135 **Fig. 2 Conformation of a polymer adsorbed onto the surface of a mineral particle**

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1138 **Fig. 3 Metabolic pathway of AMD degradation in Enterobacter aerogenes proposed by**  
1139 **Charoenpanich and Tani (2014).**

1140

1141 **Table 1 Aerobic biodegradation of acrylamide in various environmental samples**

References	Acrylamide concentration	Temperature	Lag time	Time for complete AMD degradation
Labahn <i>et al.</i> (2010)	50 µg.L <sup>-1</sup>	15°C	No degradation observed after 72 h	
		25°C	0-12h	24 h
Croll (1973)	10 µg.L <sup>-1</sup>	Non indicated	5h-50h	50h-300h
Brown (1979)	5 mg.L <sup>-1</sup>	20°C	75h	125 h
Vers (1999)	1,5-2,5 mg.L <sup>-1</sup>	Non indicated	0h	from 480 h to more than 1100 h

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1144 **Table 2 Molecular weight, pH and temperature optima of amidases purified from AMD-**  
 1145 **degrading bacteria.**

Bacterial strains	Molecular weight (kDa)	Optimum pH	Optimum temperature	Reference
<i>Geobacillus thermoglucosidasius</i> AUT-01	38.0	6.2	70°C	Cha and Chambliss, 2013
<i>Geobacillus pallidus</i> BTP-5x MTCC 9225	38.5	8.0	60°C	Sharma <i>et al.</i> 2012
<i>Ralstonia eutropha</i> AUM-01	38.0	6.3	55°C	Cha and Chambiss, 2011
<i>Variovorax boronicumulans</i> CGMCC 4969	42.0	ND*	ND*	Liu <i>et al.</i> 2013
<i>Burkholderia</i> sp. DR.Y27	47.0	8.0	40°C	Syed <i>et al.</i> 2012
<i>Rhodococcus</i> sp.	44.5	8.5	40°C	Nawaz <i>et al.</i> 1994
<i>Rhodococcus</i> sp. N-771	54.7	5.9	55°C	Ohtaki <i>et al.</i> 2010
<i>Klebsiella pneumoniae</i> NCTR 1	62.0	7.0	65°C	Nawaz <i>et al.</i> 1996
<i>Geobacillus pallidus</i> RAPc8	38.0	7.0	50°C	Makhongela <i>et al.</i> 2007
<i>Pseudonocardia thermophila</i> DSMZ 43832	52.0	7.0	70°C	Egorova <i>et al.</i> 2004
<i>Rhodococcus erythropolis</i> TA37	55.0	8.0	55°C	Lavrov <i>et al.</i> 2010

1146 \* ND: Not Determined

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