

Accepted Manuscript

This is an Accepted Manuscript of the following article:

Carlos O. Letelier-Gordo, Paulo Mira Fernandes. Coagulation of phosphorous and organic matter from marine, land-based recirculating aquaculture system effluents. *Aquacultural Engineering*. 92 (2021), 102144. ISSN 0144-8609.

The article has been published in final form by Elsevier at
<http://dx.doi.org/10.1016/j.aquaeng.2020.102144>

© 2021. This manuscript version is made available under the

CC-BY-NC-ND 4.0 license

<http://creativecommons.org/licenses/by-nc-nd/4.0/>

27 significantly affect removal of $\text{PO}_4^{3-}\text{-P}$ and TSS, but substantially increased the volume of chemical sludge
28 produced. Finally, FeCl_3 is proposed as a better overall alternative for P removal at the end-of-pipe treatment
29 in marine land-based RAS.

30 **Keywords**

31 Aluminum sulfate, Iron Chloride, Marine land-based RAS, Organic matter, Phosphorous

32

33 **Highlights**

- 34 • Coagulant utilization on the sludge flow improves P and Organic matter removal significantly
- 35 • A 90% of $\text{PO}_4^{3-}\text{-P}$ removal requires a molar ratio of 2.6:1 Fe: $\text{PO}_4^{3-}\text{-P}$ and 5.7:1 Al: $\text{PO}_4^{3-}\text{-P}$ with FeCl_3
36 and AlSO_4 , respectively
- 37 • 80% TSS removal was achieved by sedimentation and without coagulant
- 38 • Increasing coagulant dose increased the volume of chemical sludge produced
- 39 • FeCl_3 is the better alternative of the two coagulants tested for P removal in marine land-based RAS

40 **1. Introduction**

41 Phosphorous and nitrogen are the primary inorganic nutrients responsible for the eutrophication of marine
42 and fresh waters (Valsami-Jones, 2015). Eutrophication leads to an increased growth of primary production
43 and algae biomass, creating unbalanced ecosystems, degrading water quality and the ecosystem capacity,
44 depleting oxygen in the sea bottom and, finally, affecting the fisheries and tourism industry, (Henze et al.,
45 1997; WFD, 2000).

46 Recirculating aquaculture systems (RAS) are recognized as environmentally friendly as they allow the
47 reutilization of water using a series of treatments (mechanical, chemical and biological) to remove fish
48 contaminants from the rearing water (Martins et al., 2010). In the last few years, large-scale, marine, land-
49 based RAS, producing Atlantic salmon (*Salmo salar*) and yellowtail kingfish (*Seriola lalandi*), have been
50 constructed worldwide (Dalsgaard, 2017). As the industry develops and more sites appear worldwide, stricter
51 environmental regulations will increase the pressure on treating the effluents from these farms. An

52 approximation of the production cycle states that, to produce 1000 ton of fish per year, approximately 3.0 ton
53 of feed per day is required. At a feed conversion ratio (FCR) of 1.1 and 1% feed waste, the system would
54 discharge daily ca. 35 kg of total phosphorus (TP) and 877 kg of total chemical oxygen demand (TCOD)
55 (Dalsgaard and Pedersen, 2016). Most farms will have two types of effluents with different characteristics: a
56 high solids-containing effluent (drum filter and biofilter backwash) and a low solids-containing effluent
57 (overflow from water exchange), each requiring different treatments before being released to the environment.

58 Coagulation processes with metal salt agents such as aluminum or ferric chloride are standard techniques
59 in the wastewater industry for chemically removing phosphorous and suspended solids from effluents
60 (Cheremisinoff, 2002; Crittenden et al., 2012; Tchobanoglous et al., 2002). Two different action mechanisms
61 are involved in the removal process. In phosphorous removal, the addition of metal salts results in the
62 transformation of dissolved orthophosphates into particulate forms by producing chemical precipitates of low
63 solubility. The resulting precipitates and particulate phosphates are posteriorly removed throughout solids
64 separation processes, such as sedimentation, flotation and filtration (Tchobanoglous et al., 2002; Valsami-
65 Jones, 2015). The removal of suspended solids consists in the reduction or neutralization of the ζ -potential
66 (electrical double layer), promoting the interaction between particles to form larger aggregates defined as flocs
67 (Letterman et al., 1999). The ζ -potential, a property inherent to the particle electrical double layer, is a cloud
68 of ions surrounding the particle that satisfy electro neutrality, and is the main reason why these particles remain
69 in aqueous suspension for long periods of time without aggregating (Weiner and Matthews, 2003). From the
70 many factors that affect double-layer thickness, ionic strength is perhaps the most important (Letterman et al.,
71 1999). As the ionic strength of a solution increases, the extent of the double layer decreases, which in turn
72 reduces the zeta potential. For example, in seawater, the ζ -potential is 0.70 M, and in river water 0.0017 M
73 (Nazaroff & Cohen, 2001).

74 The compression of the double layer approximates particles to each other, enough for van der Waals-type
75 interaction and attachment through the aid of Brownian motion (Crittenden et al., 2012; Henze et al., 1997).
76 Coagulation applied on desalinization processes have proven that in salt water the higher ionic strength (cations
77 and anions) facilitates the general coagulation process, making it more efficient as compared to coagulation in
78 freshwater (Duan et al., 2002).

79 In aquaculture, removal of phosphorus and TSS by chemical coagulation has been studied in freshwater
80 (0 ppt) (Ebeling et al., 2006, 2003) and in brackish water (17 ppt) (Zhang et al., 2014). Information on how
81 these coagulants perform on the removal of phosphorus, total suspended solids and production of chemical
82 sludge in saltwater (>30 ppt) is still lacking. This knowledge gap is concerning, especially when the
83 construction of marine, land-based RAS is increasing.

84 The following study is aimed at determining 1) in which effluent from a marine, land-based RAS the
85 chemical agents should be applied 2) the effect of each chemical coagulant on the removal of P, organic matter,
86 TSS, and on the production of chemical sludge. To answer the above questions, different doses of AlSO_4 and
87 FeCl_3 were systematically evaluated using a modified jar test with samples from the two effluent streams
88 produced from a marine land-based RAS facility.

89

90 **2 Materials and methods**

91 2.1 Characterization of the waste masses discharged from the marine land-based RAS 92 effluents.

93 The two effluent streams from a salmon (*Salmo salar*), marine, land-based RAS (30 ppt salinity), operated at
94 1.4 kg feed/m³ of make-up water, were characterized in this trial: 1) the “sludge flow”, the effluent stream
95 containing the backwash of the drum filters and biofilters, and 2) the “overflow”, a clearer water coming from
96 the water exchange of the system. Pooled hourly samples were collected in 24-h periods every third day over
97 two months (n = 30) from both waste streams. All samples were obtained with automatic portable samplers
98 (Glacier ISCO, Teledyne, USA) and refrigerated at 4°C before transfer to the laboratory.

99 2.2 Coagulant and modified jar test description

100 Two common coagulants used in wastewater treatment were tested: FeCl_3 14% (S.Sørensen, Thisted,
101 Denmark) and $\text{AlSO}_4 \cdot 14\text{-hydrate}$ (VWR chemicals, Belgium) for evaluating the coagulation process towards
102 the removal of $\text{PO}_4^{3-}\text{-P}$, TSS, TP, TCOD and the production of chemical sludge, measured as settle sludge
103 volume final (SSV_f). The evaluation was done using a modified jar test procedure (Vandamme et al., 2010),
104 conducted in 1 L graduated tubes (Duran®, Germany) and magnetic stirrers (Big Squid, IKA, Germany).

105 Different dosing levels of coagulant (1, 2, 4 and 8 mol of coagulant:mol of $\text{PO}_4^{3-}\text{-P}$) plus a positive control (no
106 coagulant, only stirring) and a negative control “C” (no stirring) were evaluated in replicates ($n=3$). The jar
107 test protocol consists of three phases: 1) a flash mix, where coagulant and the solution are mixed at high
108 rotation (5 min at 500 rpm); 2) followed by a slow mix at lower speed (25 min at 250 rpm); 3) and finally a
109 quiescent settling, where the samples are transferred to 1 L graduated cylinders and allowed to settle (30 min).
110 Homogeneous mixed samples were collected before the addition of coagulant and at the 80% upper height of
111 the jar test tube at the end of the quiescent settling period (1 h after coagulant addition).
112 The theoretical molar doses were calculated based on average of the measurements done over the 2-month
113 period, as described above. The option to use TP over $\text{PO}_4^{3-}\text{-P}$ to achieve the molar ratios in the sludge flow
114 was considered, but not utilized, in order to achieve similar absolute dosing concentrations of FeCl_3 and
115 AlSO_4 in both waste streams.

116 2.3 Analytical methods

117 Samples for reactive phosphorous, expressed as $\text{PO}_4^{3-}\text{-P}$, were filtered through 0.2- μm syringe filters
118 (Filtropour S, SARSTEDT, Germany) and analyzed using Ion Chromatography (930 Compact IC Flex 1 with
119 a Metrosep A Supp 7 -250/4.0 column, combined with a 887 Professional UV/VIS detector; Metrohm,
120 Sweden) using 0.1 M H_2SO_4 as suppressor and 3.6 mM Na_2CO_3 as eluent. Raw samples for TCOD and TP
121 were preserved by adding 1% v/v sulfuric acid (4 M H_2SO_4 , Merck Millipore, Germany), and maintained at
122 4°C until further analysis. Determination of TCOD was performed using digestion vials (LCK 514, Hach
123 Lange, Germany) and TP was determined according to ISO, 6491 (DS, 1998). Total suspended solids (TSS)
124 were determined according to 2440D (Eaton et al., 1995) and final settled sludge volume (SSV_f) according to
125 2710D (Eaton et al., 1995).

127 2.4 Data treatment

128 The calculation of the discharged masses from the RAS and the removal efficiency (η) of the tested parameters
129 from the Jar test was determined following equations 1 and 2, respectively.

130

131 Mass produced per day (g/d) = Concentration of parameter (g/m³) * Flow (m³/d) (Eq. 1)

132

133
$$\eta (\%) = \frac{C_i - C_f}{C_i} * 100$$
 (Eq. 2)

134 Where:

135 C_i: concentration of the parameter tested in raw samples

136 C_f: concentration of the parameter after the jar test procedure.

137 When η is positive it means removal of substance while a negative values means accumulation.

138

139 The supersaturation ratio (S) (Valsami-Jones, 2015), or the fraction of reactive phosphorus in solution, was

140 calculated accordingly:

141

142
$$S = \frac{PO_4^{3-}-P}{TP}$$
 (Eq. 3)

143 Where:

144 PO₄³⁻-P: mass of PO₄³⁻-P (mg P/L)

145 TP: mass of Total phosphorous (mg P/L)

146

147 2.5 Statistical analysis

148 Differences in the removal efficiencies for PO₄³⁻-P, TSS, TCOD, TP and settled sludge volume final (SSVf)

149 between different coagulant dosing and control were examined by one-way ANOVA analysis, followed by

150 Tukey–Kramer multiple comparison of means test, with a 95% family-wise confidence level. Differences were

151 considered significant when P < 0.05, and values are stated as the mean ± standard deviation (SD). The

152 statistical analyses were carried out using the R software (R CoreTeam, 2016).

153

154 3 Results

155 3.1 Masses discharged from the RAS effluents

156 The RAS facility discharges of the end-of-pipe treatment phase a total flow of 2400 m³/d, divided into two
157 streams: the sludge flow (480 m³/d) and the overflow (1920 m³/d) (Table 1). The overflow, 80% of the total
158 discharged flow, contained the majority of the dissolved phosphorous (66% total mass discharged) with a
159 supersaturation ratio (S) of 0.79 (Table 1). On the other hand, the sludge flow, 20% of the total discharged
160 flow, contained the majority of the daily masses discharged from the RAS: 82% TCOD, 99% TSS and 90%
161 TP (Table 1).

162

163 Table 1. RAS overflow and sludge flow daily discharged masses. Each parameter is presented as concentration
164 in the water, daily masses (mass estimated according to Eq. 1), and the fraction of the parameter from the total
165 discharged mass fraction (n=30 ± S.D.).

166

167 3.2 Effect of chemical coagulant dosing on the removal of P, TSS, TCOD, and SSV_f

168 production in the sludge flow.

169 The coagulant dosing level significantly affected PO₄³⁻-P removal, while settling or stirring of the solution
170 (negative control and control, respectively) had only a minimal effect on these parameters (5%-16%) (Fig. 1).

171 According to the equations obtained from the response dose towards PO₄³⁻-P removal, 90% removal can be
172 achieved when applying FeCl₃ at molar ratio of 2.6:1 Fe: PO₄³⁻-P (P=0.003) or AlSO₄ at molar ratio of 5.7:1
173 Al: PO₄³⁻-P (P<0.001).

174 For TSS, settling without coagulant addition “C” (negative control) already removed 79-81 % of TSS. Stirring
175 (positive control) improved further the overall TSS removal efficiency (81-86%), but not significantly
176 (P=0.171). The TSS removal was further improved, when the coagulants were applied at molar ratios above
177 1.3 Fe: PO₄³⁻-P (P=0.001) or 7.8 Al: PO₄³⁻-P (P <0.001) (Fig 1).

178 The amount of settled sludge, expressed as SSV_f, did not change significantly between the positive or negative
179 controls. However, SSV_f increased significantly as the coagulant dose increased, reaching a 94% increment
180 with FeCl₃ at a molar ratio of 10.40 Fe: PO₄³⁻-P (P<0.001), and a 50% increase for AlSO₄ at a molar ratio of
181 7.8:1 Al: PO₄³⁻-P (P<0.001).

182 The removal of organic matter, measured as TCOD, was between 57-67% for the positive and the negative
183 control (stirring and settling, respectively). Increasing the coagulant dose improved significantly the TCOD
184 removal efficiency: 84% for FeCl₃ at a molar ratio of 10.40 Fe: PO₄³⁻-P (P<0.001) and 72% for AlSO₄ at a
185 molar ratio of 7.8:1 Al: PO₄³⁻-P (P=0.001) (Fig 1).

186 TP removal was similar to that of TCOD: between 76-84% with settling or only stirring (negative and positive
187 control, respectively). Increasing the coagulant dose increased the removal efficiency up to 95% for FeCl₃ at
188 a molar ratio of 10.40 Fe: PO₄³⁻-P (P<0.001) and above 92% for AlSO₄ at a molar ratio higher than 7.8:1 Al:
189 PO₄³⁻-P (P<0.001) (Fig 1).

190

191 Figure 1: Effect of different molar doses of FeCl₃ (right-side figures) and AlSO₄ (left-side figures) on the
192 removal of PO₄³⁻-P, TP, TSS and production of sludge, expressed as SSV_f, in the sludge flow stream using a
193 modified Jar test method (mean ± SD, n=3). The residual concentration of the observed parameter is presented
194 on the Y-left axis, the observed parameter on the Y-right axis, and different applied coagulant doses on the X-
195 axis. C stands for the negative control (settling, no coagulant addition) and 0 correspond to positive control
196 (stirring). Removal efficiencies expressed in % are calculated in respect to initial concentrations (t=0), as
197 described in Eq. 1.

198

199 3.3 Effect of chemical coagulant dosing on the removal of P, TSS, TCOD, and SSV_f
200 production in the overflow stream.

201 For the overflow stream, the coagulant dose significantly affected removal on PO₄³⁻-P, while the action of
202 settling (negative control) and stirring (control) had a minimal effect on PO₄³⁻-P removal (1%-2%) (Figure 2).
203 A 90% removal of PO₄³⁻-P was achieved at 5.4 Fe:PO₄³⁻-P (P<0.001) and 8.9 Al:PO₄³⁻-P (P<0.001) and TP
204 removal improved significantly when coagulant dose was increased, reaching 39% removal with FeCl₃ at a
205 molar ratio of 10.40:1 Fe: PO₄³⁻-P (P<0.001), and 16% with AlSO₄ at a molar ratio of 9.69 Al: PO₄³⁻-P
206 (P<0.001). The positive (stirred) and negative control (settled), showed a removal efficiency between 0 - 2%
207 (Fig 2).

208 A 25% removal of organic matter (TCOD) was achieved with FeCl_3 at 10.4:1 Fe: $\text{PO}_4^{3-}\text{-P}$ ($P < 0.014$), while
209 AlSO_4 had no effect on TCOD removal ($P = 0.645$). Increasing the coagulant dose, significantly increased the
210 production of TSS, with a 2.2 fold production with FeCl_3 at a molar ratio of 10.40:1 Fe: $\text{PO}_4^{3-}\text{-P}$ ($P < 0.001$) and
211 a 13.4 fold production of TSS with AlSO_4 at a molar ratio of 9.69:1 Al: $\text{PO}_4^{3-}\text{-P}$ ($P < 0.001$) (Figure 2). Due to
212 the low amount of solids contained in the overflow stream, it was not possible to record SSV_f .
213 Figure 2: Effect different doses of FeCl_3 (right-side figures) and AlSO_4 (left-side figures) on the removal of
214 $\text{PO}_4^{3-}\text{-P}$, TP and TSS in the overflow stream using a modified Jar test method (mean \pm SD, $n = 3$). The residual
215 concentration of the observed parameter is presented on the Y-left axis, the observed parameter on the Y-right
216 axis, and the applied coagulant molar doses on the X-axis. C stands for the negative control (settling) and 0
217 correspond to positive control (stirring). Removal efficiencies expressed in % are calculated in respect to initial
218 concentrations ($t = 0$), according to Eq. 1.

219

220 4 Discussion

221 Previous waste characterizations from RAS systems (Dalsgaard and Pedersen, 2011), stated that more than
222 85% of P discharged from fish farms is solid-bound. In this study, 90%, 99% and 82% of TP, TSS and TCOD
223 masses, respectively, were observed in the sludge flow, while the majority (66%) of $\text{PO}_4^{3-}\text{-P}$ mass was observed
224 in the overflow. Because of the greater initial mass content, the highest removal efficiency for TP, TSS and
225 TCOD were achieved when targeting the treatment on the sludge flow.

226 When applying FeCl_3 and AlSO_4 in the sludge flow and considering 90% of $\text{PO}_4^{3-}\text{-P}$ removal as a comparative
227 baseline, it was found that FeCl_3 requires a lower molar ratio dose than AlSO_4 (2.6 vs 5.7 mol flocculant: mol
228 $\text{PO}_4^{3-}\text{-P}$). According to the stoichiometry of the applied coagulants on P removal (Tchobanoglous et al., 2002),
229 to remove one gram of P, approximately 2.9 g of FeCl_3 or 3.7 g of AlSO_4 is needed. However, in reality,
230 colloidal suspensions and specific cations and anions in water will influence the stoichiometric interaction
231 between FeCl_3 and AlSO_4 with P, potentially requiring different dosing levels (Duan et al., 2002; Henze et al.,
232 1997). In aquaculture, 80-90 mg/L of FeCl_3 has been found to remove up to 90% of $\text{PO}_4^{3-}\text{-P}$ in freshwater
233 systems (~10:1 Fe: $\text{PO}_4^{3-}\text{-P}$ and 5.65 Al: $\text{PO}_4^{3-}\text{-P}$ molar ratio) (Ebeling et al., 2003). Similarly, in brackish

234 water (17 ppt) 27.6 mg of Fe/L (FeCl_3) and 12.6 mg Al/L of polymeric aluminum were needed to achieve 90%
235 PO_4^{3-} -P removal (2.6 Fe: PO_4^{3-} -P and 5.63 of Al: PO_4^{3-} -P molar ratio) (Zhang et al., 2014). It seems that there
236 is an interaction effect of higher water ionic strengths on removal of P, but that this effect stabilizes beyond 17
237 ppt. It also seems that salinity greatly improves the interaction between Fe^{3+} and P, but not the removal of P
238 by Al^{2+} . Comparatively, the sludge samples in this trial had a potentially higher organic carbon content than in
239 Zhang paper. This could help explain why high salinity in our samples only partially improved the removal of
240 P by chemical flocculation (Fettig and Ratnaweera, 1993).

241 To standardize the application of coagulant to a relatable number, and based on the model by Dalsgaard &
242 Pedersen (2011) and on the fractions of phosphorus observed in this study, the amount of coagulant required
243 to remove 90% of PO_4^{3-} -P discharged from the RAS sludge flow is around 11.2 kg Fe(III)/ton feed, and 11.9
244 kg Al/ton feed.

245 When applying a dose of FeCl_3 and AlSO_4 (2.6 vs 5.7 mol flocculant: mol PO_4^{3-} -P, respectively), we achieved
246 high removal of TSS (90-91%) and TP (91%). However, approximately 40% excess sludge with either
247 coagulant was produced, suggesting that coagulant overdosing clearly increases the amount of chemical
248 sludge, and only marginally improves the removal efficiencies of TSS and PO_4^{3-} -P.

249 A high removal of TSS (between 80-86%) in this study was achieved by either settling or solely stirring the
250 solution (negative and positive control). In other studies, practically no removal of TSS was found without
251 coagulant addition (Ebeling et al., 2003; Zhang et al., 2014). We suggest that settling should always be the
252 primary step in fresh or seawater flocculation processes, but salinity also has a direct effect on TSS removal.

253 A higher salinity exhibits a higher ionic strength, reducing the double-layer thickness of particles, resulting in
254 a lower electrical repulsive force which, in this way, improves the coagulation settling, and overall TSS
255 removal process (Crittenden et al., 2012; Henze et al., 1997). Furthermore, we found that increasing the doses
256 of AlSO_4 and FeCl_3 did not significantly improve TSS removal. On the contrary, the volume of chemical
257 sludge (SSV_f) was increased 50 and 93%, for FeCl_3 and AlSO_4 , respectively. This is an important finding, as
258 the effect that these coagulants have on the removal of TSS at the test salinity is marginal when overdosing,
259 compared to the side effects of increased chemical sludge volume that will need to be disposed.

260 Considering the sludge flow organic fraction showed a high settleability (80-84%) and 90% of TP is discharged
261 through the sludge flow, we propose to take advantage of this situation and apply a pre-settling treatment
262 before any coagulant addition. Being the overall TP removal approximately 72-75% (10.3 - 10.8 Kg/d) of the
263 total P produced from the farm (14.4 Kg/d), settling the sludge would decrease the amount of coagulant
264 required, since the less dense effluent could marginally improve removal efficiencies through coagulation with
265 chemical agents. It would further reduce the amount of chemical sludge produced and the impact that metal
266 ions have on posterior thickening and dewatering of the sludge (Turovskiy and Mathai, 2006), thus, improving
267 the overall cost-effectiveness of the treatment.

268 In general, both coagulants improved PO_4^{3-} -P removal while having a minor effect on TSS removal. FeCl_3
269 achieved better removal efficiencies at a lower molar dose, but produced more chemical sludge, indicating
270 that when choosing a flocculant, other collateral effects must also be considered. Aluminium is known as a
271 powerful neurotoxicant to humans and other animals (Flaten, 2001; Igbokwe et al., 2020), and deteriorates
272 the environment (Jaishankar et al., 2014). Additionally, higher content of sulfate in seawater, might promote
273 H_2S production in sludge basins, and, while FeCl_3 has been shown to suppress H_2S production (Zhang et al.,
274 2008), AlSO_4 could be a the precursor of H_2S due to addition of the SO_4^{2-} ion (Letelier-Gordo et al., 2020).
275 Therefore, FeCl_3 should be to consider as coagulant more appropriate to be used in marine water end-of-pipe
276 treatment.

277 **5 Conclusions**

278 In the removal of P as end-of-pipe treatment in a marine land-based RAS, major efforts must be focused on
279 treating the effluent stream from the backwash of the drum filters and biofilters backwash (sludge flow), as
280 basically 90% of TP, 99% of TSS and 82% of TCOD are discharged through this effluent. To remove 90% of
281 PO_4^{3-} -P, FeCl_3 requires a molar ratio dose of 2.6 mol $\text{Fe}:\text{PO}_4^{3-}$ while AlSO_4 requires a molar ratio of 5.7 mol
282 $\text{Al}:\text{PO}_4^{3-}$ -P. Both coagulants showed a low response for TSS removal, whereas the controls (settling and
283 stirring) showed already an 80-86% removal. Overdosing of coagulant beyond the recommended dose will
284 have minimal effects on PO_4^{3-} -P and TSS removal, while highly affecting the volume of chemical sludge
285 produced which requires further treatment. Finally, FeCl_3 is suggested as a better overall alternative to be used

286 at the end-of-pipe treatment in marine land-based RAS, due to the higher efficiency at lower molar doses, and
287 the potential harmfulness of $AlSO_4$ to receiving water ecosystems due to addition of both Al^{2+} and SO_4^{2-} and
288 their potential toxic reactions.

289

290 **6 Acknowledgements**

291 This research was funded by The Ministry of Food, Agriculture and Fisheries of Denmark through the project
292 WASTETREAT (GUDP 39190) “Cost effective solutions for End-of-pipe treatment in salt water RAS”.

293

294 **7 References**

295

- 296 Cheremisinoff, N., 2002. Handbook, 2002, Handbook of Water and Wastewater Treatment Technology,
297 Nicholas P. Cheremisinoff. Butterwoth-Heinemann.
- 298 Crittenden, J.C., Trussell, R.R., Hand, D.W., Howe, K.J., Tchobanoglous, G., 2012. Water Treatment:
299 Principles and Design. John Wiley & Sons, Inc.
- 300 Dalsgaard, J., 2017. 4th NordicRAS Workshop on Recirculating Aquaculture Systems Aalborg, Denmark, 12-
301 13 October 2017. Book of Abstracts. National Institute of Aquatic Resources, Hirtshals, Denmark.
- 302 Dalsgaard, J., Pedersen, P.B., 2016. Waste production and fish farm model: Manual and model
303 assumptions. DTU Aqua-rapport nr. 309-2016, Technical University of Denmark.
- 304 Dalsgaard, J., Pedersen, P.B., 2011. Solid and suspended/dissolved waste (N, P, O) from rainbow trout
305 (*Oncorhynchus mykiss*). *Aquaculture* 313, 92–99. <https://doi.org/10.1016/j.aquaculture.2011.01.037>
- 306 Duan, J., Wang, J., Graham, N., Wilson, F., 2002. Coagulation of humic acid by aluminium sulphate in saline
307 water conditions. *Desalination* 150, 1–14. [https://doi.org/10.1016/S0011-9164\(02\)00925-6](https://doi.org/10.1016/S0011-9164(02)00925-6)
- 308 Eaton, A., Clesceri, L., Greenberg, A., 1995. Standard Methods for the Examination of Water and
309 Wastewater. APHA.
- 310 Ebeling, J.M., Sibrell, P.L., Ogden, S.R., Summerfelt, S.T., 2003. Evaluation of chemical coagulation-
311 flocculation aids for the removal of suspended solids and phosphorus from intensive recirculating
312 aquaculture effluent discharge. *Aquac. Eng.* 29, 23–42. [https://doi.org/10.1016/S0144-
313 8609\(03\)00029-3](https://doi.org/10.1016/S0144-8609(03)00029-3)
- 314 Ebeling, J.M., Welsh, C.F., Rishel, K.L., 2006. Performance evaluation of an inclined belt filter using
315 coagulation/flocculation aids for the removal of suspended solids and phosphorus from microscreen
316 backwash effluent. *Aquac. Eng.* 35, 61–77. <https://doi.org/10.1016/j.aquaeng.2005.08.006>
- 317 Fettig, J., Ratnaweera, H., 1993. Influence of dissolved organic matter on coagulation/ flocculation of
318 wastewater by alum. *Water Sci. Technol.* 27, 103–112. <https://doi.org/10.2166/wst.1993.0269>

- 319 Flaten, T.P., 2001. Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. *Brain*
320 *Res. Bull.* 55, 187–196. [https://doi.org/10.1016/S0361-9230\(01\)00459-2](https://doi.org/10.1016/S0361-9230(01)00459-2)
- 321 Henze, M., Harremoës, P., La Cour, J., Arvin, E., 1997. *Wastewater treatment. Biological; and chemical*
322 *processes.* Springer-Verlag Berlin Heidelberg, New York, NY, USA.
323 <https://doi.org/10.1017/S0025315400038911>
- 324 Igbokwe, I.O., Igwenagu, E., Igbokwe, N.A., 2020. Aluminium toxicosis: A review of toxic actions and effects.
325 *Interdiscip. Toxicol.* 12, 45–70. <https://doi.org/10.2478/intox-2019-0007>
- 326 Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and
327 health effects of some heavy metals. *Interdiscip. Toxicol.* 7, 60–72. [https://doi.org/10.2478/intox-](https://doi.org/10.2478/intox-2014-0009)
328 [2014-0009](https://doi.org/10.2478/intox-2014-0009)
- 329 Letelier-Gordo, C.O., Aalto, S.L., Suurnäkki, S., Bovbjerg, P., 2020. Aquacultural Engineering Increased
330 sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste. *Aquac.*
331 *Eng.* 89, 8. <https://doi.org/10.1016/j.aquaeng.2020.102062>
- 332 Letterman, R.D., Amirtharajah, A., Melia, C.R.O., 1999. *Water Quality and Treatment*, 5th ed. McGraw-Hill,
333 NY.
- 334 Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P.,
335 d'Orbcastel, E.R., Verreth, J. a. J., 2010. New developments in recirculating aquaculture systems in
336 Europe: A perspective on environmental sustainability. *Aquac. Eng.* 43, 83–93.
337 <https://doi.org/10.1016/j.aquaeng.2010.09.002>
- 338 Tchobanoglous, G., Burton, F., Stensel, H.D., 2002. *Wastewater Engineering: Treatment and Reuse/Metcalf*
339 *& Eddy, Inc.* McGraw-Hill.
- 340 Turovskiy, I.S., Mathai, P.K., 2006. Thickening and Dewatering. *Wastewater Sludge Process.* 60–135.
341 <https://doi.org/10.1002/047179161x.ch3>
- 342 Valsami-Jones, E., 2015. Phosphorus in Environmental Technology: Principles and Applications 4, 656.
343 <https://doi.org/10.2166/9781780402758>
- 344 Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K., 2010. Flocculation of microalgae using cationic
345 starch. *J. Appl. Phycol.* 22, 525–530. <https://doi.org/10.1007/s10811-009-9488-8>
- 346 Weiner, R.F., Matthews, R.A., 2003. *Environmental Engineering: Fourth Edition*, *Environmental Engineering:*
347 *Fourth Edition.* <https://doi.org/10.1016/B978-0-7506-7294-8.X5000-3>
- 348 WFD, 2000. Directive of the European Parliament and of the Council 2000/60/EC. Establishing a Framework
349 for Community Action in the Field of Water Policy. *Off. J. Eur. Parliam.* L327, 1–82.
350 <https://doi.org/10.1039/ap9842100196>
- 351 Zhang, L., De Schryver, P., De Gussemé, B., De Muynck, W., Boon, N., Verstraete, W., 2008. Chemical and
352 biological technologies for hydrogen sulfide emission control in sewer systems: A review. *Water Res.*
353 42, 1–12. <https://doi.org/10.1016/j.watres.2007.07.013>
- 354 Zhang, X., Hu, J., Spanjers, H., Van Lier, J.B., 2014. Performance of inorganic coagulants in treatment of
355 backwash waters from a brackish aquaculture recirculation system and digestibility of salty sludge.
356 *Aquac. Eng.* 61, 9–16. <https://doi.org/10.1016/j.aquaeng.2014.05.005>

a modified Jar test method (mean \pm SD, n=3). The residual concentration of the observed parameter is presented on the Y-left axis, the observed parameter on the Y-right axis, and different applied coagulant doses on the X-axis. C stands for the negative control (settling, no coagulant addition) and 0 correspond to positive control (stirring). Removal efficiencies expressed in % are calculated in respect to initial concentrations ($t=0$), as described in Eq. 1.

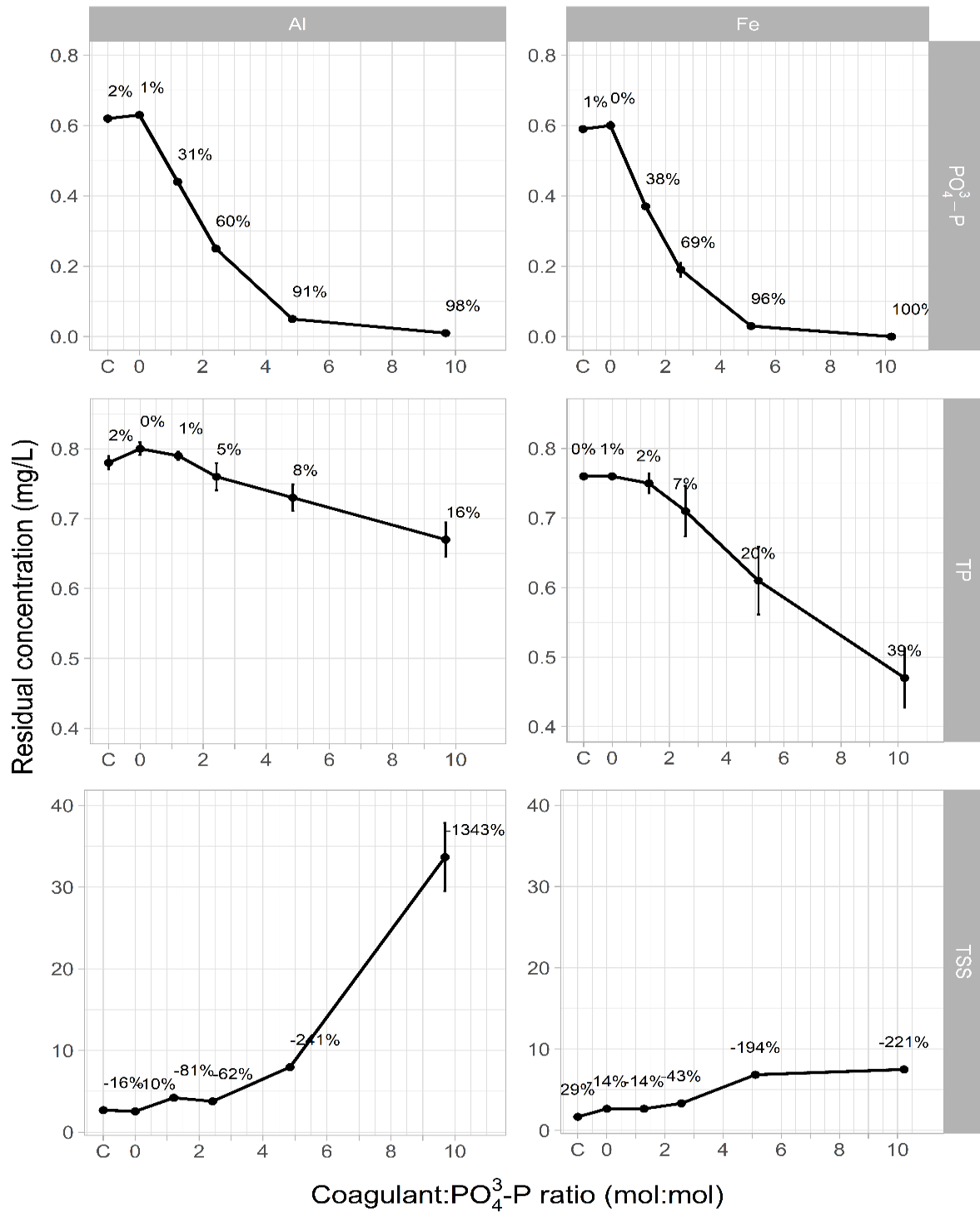


Figure 2: Effect different doses of FeCl_3 (right-side figures) and AlSO_4 (left-side figures) on the removal of $\text{PO}_4^{3-}\text{-P}$, TP and TSS in the overflow stream using a modified Jar test method (mean \pm SD, n=3). The residual concentration of the observed parameter is presented on the Y-left axis, the observed parameter on the Y-right axis, and the applied coagulant molar doses on the X-axis. C stands for the negative control (settling) and 0 correspond to positive control (stirring). Removal efficiencies expressed in % are calculated in respect to initial concentrations (t=0), according to Eq. 1.

Table 1. RAS overflow and sludge flow daily discharged masses. Each parameter is presented as concentration in the water, daily masses (mass estimated according to Eq. 1), and the fraction of the parameter from the total discharged mass fraction ($n=30 \pm \text{S.D.}$).

Location	RAS overflow			Sludge flow			
	Parameter	Conc. ^a	Mass ^b	Fraction ^c	Conc. ^a	Mass ^b	Fraction ^c
Daily flow (m ³ /d)		1920		80%	480		20%
pH		7.51-7.68			7.02-7.29		
TCOD		72.5±13.4	139±26	18%	1345±176	646±85	82%
TSS		2.28±0.14	4.38±0.27	1%	962±39	462±19	99%
TP		0.78±0.02	1.50±0.04	10%	26.9±1.47	12.9±0.7	90%
PO ₄ ³⁻ -P		0.62±0.02	1.19±0.04	66%	1.27±0.20	0.61±0.10	34%
S (PO ₄ ³⁻ -P/TP)		0.79±0.03			0.05±0.01		

^a – concentrations as mg/L for TCOD, TSS, TP and PO₄³⁻-P.

^b – masses as kg/d for TCOD, TSS, TP and PO₄³⁻-P.

^c – fraction of total daily waste (by mass) contained in the RAS overflow, or sludge flow.