

1     **Maximisation of the organic load rate and minimisation of oxygen consumption in**  
2     **aerobic biological wastewater treatment processes by manipulation of the hydraulic**  
3                                     **and solids residence time**

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5     **Abstract**

6     A systematic experimental study of the effect of hydraulic residence time (HRT) and  
7     solids residence time (SRT) on conventional suspended-growth biological wastewater  
8     treatment processes was carried out. The aim of this study was to identify the  
9     conditions that minimise the reactor volume, i.e. maximise the organic load rate (OLR),  
10    and minimise the oxygen consumption. Lab-scale sequencing batch reactors (SBRs)  
11    were operated with glucose or ethanol as only carbon sources, with HRT in the range  
12    0.25-4 day and SRT in the range 1-71 day. The highest OLR values which gave  
13    satisfactory performance were 4.28 and 4.14 gCOD/l.day for glucose and ethanol,  
14    respectively, which are among the highest reported for conventional aerobic  
15    suspended-growth processes. The highest OLR values were obtained with HRT=0.25  
16    day, SRT=3.1 day for glucose and HRT=0.5 day, SRT=4.9 day for ethanol. The minimum  
17    oxygen consumption was 0.36 and 0.69 kg O<sub>2</sub>/kg COD removed for glucose and  
18    ethanol, respectively. In disagreement with conventional theories, it was found that  
19    biomass production also depended on the OLR as well as on the SRT, higher OLRs  
20    giving lower biomass production for the same SRT. From the kinetic analysis of the  
21    experimental data, this behaviour, which has important consequences for the design  
22    of biological wastewater treatment processes, was explained with a higher rate of  
23    endogenous metabolism at higher OLRs.

24    **Keywords:** Aerobic wastewater treatment; hydraulic residence time (HRT); organic

25 load rate (OLR); solids residence time (SRT); oxygen consumption.

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## 29 **1. Introduction**

30 The aim of aerobic biological wastewater treatment processes is to treat the influent  
31 wastewater with the highest possible reduction of the COD and BOD, with the  
32 minimum possible size of the reaction tank and the minimum possible oxygen  
33 consumption. A high COD reduction is required to maintain the high environmental  
34 quality of the receiving water body, a small volume of the reaction tank decreases the  
35 capital costs and the land usage by the plant, low oxygen consumption minimises the  
36 energy costs and the environmental footprint of the plant. In addition, the production  
37 of waste sludge needs also to be taken into account in the design of biological  
38 treatment processes. Usually, waste sludge is considered a liability which needs to be  
39 minimised, but the increasing use of anaerobic digestion to convert sludge into  
40 methane is showing that waste sludge can rather be seen as a resource (McCarty et al.,  
41 2011).

42 As far as the reactor volume is concerned, for a given flow rate and composition of the  
43 influent wastewater, smaller reactor volumes correspond to lower values of the  
44 hydraulic residence time (HRT) and, correspondingly, to higher values of the  
45 volumetric organic load rate (OLR). In conventional suspended-growth activated  
46 sludge processes, the OLR is typically in the range 0.5-1.5 kg COD/m<sup>3</sup>.day (WEF, 2012).  
47 Various technologies have been investigated to increase the OLR and therefore  
48 decrease the reactor volume, e.g. air-bubble or jet-loop bioreactors, membrane  
49 bioreactors or granular sludge. For example, Petruccioli et al. (2000) reported the  
50 treatment of winery wastewaters in an air-bubble column bioreactor at organic loads  
51 up to 8.8 g COD/l.day, and Bloor et al. (1995) reported treatment of a brewery  
52 wastewater in a jet loop reactor at organic loads up to 50 g COD/l.day. Holler and

53 Trosch (2001) reported successful operation of membrane bioreactors with OLRs of up  
54 to 13 g COD/l.day. Liu and Tay (2015) operated aerobic granular reactors with a long-  
55 term stable performance at the OLR of 6 g COD/l.day. Although these technologies  
56 have been proven successful and are used at full scale, they also have disadvantages  
57 and are not always applicable, e.g. membrane bioreactors are subject to fouling and  
58 are often expensive and the mechanism of aerobic granulation is not yet completely  
59 understood. Other technologies require special reactor types and aerators  
60 configurations which are not of general applicability in activated sludge processes.

61 The maximum OLR that can possibly be achieved in conventional suspended-growth  
62 biological processes is limited by the maximum biomass concentration that can be  
63 maintained in the biological reactor, which is in turn limited by the negative effect of  
64 high biomass concentrations on the aeration efficiency and on the settling rate.  
65 However, the biomass concentration also depends on the solids residence time (SRT)  
66 and it is therefore conceivable that SRT and HRT might be optimised together to  
67 maximise the OLR while still maintaining a biomass concentration that is not too high.  
68 In this optimisation, it has to be taken into account that the SRT determines the  
69 effluent substrate concentration, the oxygen consumption and the biomass production  
70 in the plant (Grady et al., 2011; Dionisi, 2017). In summary, the design parameters HRT  
71 and SRT need to be chosen to satisfy the objectives of the highest possible effluent  
72 quality, lowest reactor volume and lowest oxygen consumption.

73 Typically conventional suspended-growth activated sludge processes for carbon  
74 removal are operated with values of the SRT in the range 3-15 days (Grady et al., 2011;  
75 WEF, 2012). However, recent studies (Jimenez et al., 2015) on the high-rate activated  
76 sludge process (HRAS) have shown that efficient COD removal can be obtained even at

77 **SRT lower than 2 days.** A study by Ge et al. (2013) has shown, with a slaughterhouse  
78 wastewater, that activated sludge processes can be successful even with low SRT  
79 values (2-3 days). In that study, operation at low SRT allowed the use of a short HRT  
80 and therefore a high organic load rate of up to 5.8 g COD/l.day. **These findings were**  
81 **later confirmed in another study from the same group (Ge et al., 2017) using**  
82 **wastewater effluent from a sewer biofilm reactor.** The Authors also observed a high  
83 anaerobic degradability of the produced sludge and a positive effect of lower SRT in  
84 the aerobic process on the anaerobic digestion of the sludge, an effect which was also  
85 observed by Gossett et al. (1982) and Bolzonella et al. (2005).

86 Although several studies have been reported on the effect of HRT and SRT in activated  
87 sludge processes, usually these parameters have not been optimised simultaneously  
88 for the maximisation of the OLR and the minimisation of the oxygen consumption.  
89 Furthermore, there is very little reported information on how the OLR affects the  
90 kinetic parameters of activated sludge models, in particular the parameters that  
91 mostly affect oxygen consumption and biomass production, i.e. the growth yield and  
92 the specific rate of endogenous metabolism. **A recent study by Liu and Wang (2015)**  
93 **investigated and modelled the effect of dissolved oxygen and SRT on sludge**  
94 **production, finding that low oxygen concentrations reduce the degradation of cell**  
95 **debris and therefore increase the sludge production. An experimental optimisation of**  
96 **the HRT and SRT for municipal wastewater was carried out by Jimenez et al. (2015),**  
97 **who identified  $SRT > 1.5$  days and  $HRT > 30$  min as the optimum conditions for the HRAS**  
98 **process, however they did not attempt to give a quantitative interpretation of their**  
99 **data using kinetic modelling (e.g. determining the growth yield and the rate of**  
100 **endogenous metabolism).** The effect of HRT and SRT on activated sludge process

101 performance was investigated by Barr et al. (1996) using a wastewater from Kraft mills.  
102 However, in this study the OLR was not optimised and was in all cases below 1.5  
103 kgBOD/m<sup>3</sup>.day. Surprisingly, the authors observed that BOD removal was more  
104 affected by the HRT than by the SRT. The effect of the SRT on phenol and o-cresol  
105 removal was investigated by Nakhla et al. (1994), however this study was carried out at  
106 constant HRT and OLR and the process was therefore not optimised. Both studies by  
107 Barr et al. (1996) and Nakhla et al. (1994) were carried out with potentially inhibiting  
108 wastewaters, which makes it more difficult to interpret their results in terms of  
109 optimisation of the operating parameters. **As far as nitrogen removal is concerned, the**  
110 **effect of SRT on ammonia removal and nitrate and nitrite production was investigated**  
111 **and modelled in a recent study (Liu and Wang, 2014).**

112 The aim of this study is to carry out a systematic experimental analysis of the  
113 optimisation of aerobic biological wastewater treatment processes. In particular, the  
114 aim is to identify the conditions that minimise the reactor volume and the oxygen  
115 consumption and maximise the biomass production while maintaining a satisfactory  
116 performance in terms of COD removal and biomass settling. Also, this study is aimed at  
117 determining the effect of the OLR on the biomass growth yield and on the specific rate  
118 of endogenous metabolism, which are the most important parameters in the  
119 calculation of oxygen consumption and biomass production in biological processes. In  
120 this study, we will assume that biomass production is a benefit for the process because  
121 of its potential for energy generation using anaerobic digestion. This optimisation  
122 study was carried out by running aerobic reactors at different values of HRT and SRT.  
123 The study was carried out with two synthetic wastewaters, using glucose and ethanol  
124 as only carbon sources.

## 125 2. Background theory

126 In this section we summarise the fundamental theory of activated sludge processes  
127 which is behind and has guided our experimental study. The theory in this section is  
128 adapted from our recent work (Dionisi, 2017).

129 The equations below refer to a continuous-flow activated sludge process consisting of  
130 a perfectly mixed biological reactor followed by a settling tank with biomass  
131 recirculation. We assume that the excess sludge is removed from the bottom of the  
132 settling tank. We will use the following definitions:

$$133 \quad HRT = \frac{V}{Q} \quad (1)$$

$$134 \quad SRT = \frac{VX}{Q_w X_R + (Q - Q_w) X_{eff}} \quad (2)$$

$$135 \quad OLR = \frac{QS_0}{V} \quad (3)$$

136 with the following meaning of the symbols: **HRT=hydraulic residence time (day);**  
137 **SRT=solids residence time (day); OLR=organic load rate (gCOD/l.day);**  $V$  = reactor  
138 volume (l);  $Q$  = influent wastewater flow rate (l/day);  $S_0$  = influent substrate  
139 concentration (gCOD/l);  $X$ =biomass concentration in the reactor (gVSS/l);  $X_{eff}$ = biomass  
140 concentration in the supernatant from the settling tank (gVSS/l);  $X_R$  = biomass  
141 concentration at the bottom of the settling tank and in the recycle stream (gVSS/l);  $Q_w$   
142 = sludge waste flow rate (l/day). We will assume that substrate removal and biomass  
143 growth are described by Monod kinetics with endogenous metabolism:

$$144 \quad r_x = \frac{\mu_{max} S}{K_s + S} X; r_s = -\frac{\mu_{max} S}{K_s + S} \frac{X}{Y_{X/S}}; r_{end} = -bX$$

145 with the following meaning of the symbols:  $r_x$ = biomass growth rate (gVSS/l.day);  $r_s$ =

146 substrate removal rate (gCOD/l.day);  $r_{end}$  = rate of endogenous metabolism  
 147 (gVSS/l.day).  $\mu_{max}$  ( $\text{day}^{-1}$ ),  $K_s$  (gCOD/l) and  $b$  ( $\text{day}^{-1}$ ) are kinetic parameters. In this study,  
 148 a simple model of endogenous metabolism is considered, which assumes that all the  
 149 biomass that decays is fully oxidised to carbon dioxide and water with no generation of  
 150 cell debris. More complex models of endogenous metabolism, which include the  
 151 generation of cell debris or of an endogenous residue, have also been developed  
 152 (Friedrich and Takacs, 2013; Liu and Wang, 2015; Ramdani et al., 2012).

153 With these assumptions, the relationship between effluent substrate concentration ( $S$ ,  
 154 gCOD/l), SRT and kinetic parameters is:

$$155 \quad S = \frac{bK_s SRT + K_s}{(\mu_{max} - b)SRT - 1} \quad (4)$$

156 Equation (4) shows that, for given kinetic parameters, the effluent substrate  
 157 concentration depends only on the SRT.

158 The biomass concentration in the reactor is given by:

$$159 \quad X = \frac{(S_0 - S)Y_{x/s}SRT}{(1 + b \cdot SRT)HRT} \quad (5)$$

160 Equation (5) shows that, for a given influent concentration, the biomass concentration in  
 161 the reactor depends on the SRT and on the HRT. The biomass concentration increases  
 162 by increasing the SRT and by decreasing the HRT.

163 The biomass production and the oxygen consumption per unit of influent flow rate are  
 164 given by:

$$165 \quad \frac{P_x}{Q} \left( \frac{\text{kg biomass}}{\text{day} \cdot \frac{\text{m}^3}{\text{day}}} \right) = \frac{(S_0 - S)Y_{x/s}}{1 + b \cdot SRT} \quad (6)$$

166 
$$\frac{Q_{O_2\text{biomass}}}{Q} \left( \frac{\text{kg } O_2}{\text{day} \cdot \frac{m^3}{\text{day}}} \right) = (S_0 - S) \left( 1 - \frac{1.42 \cdot Y_{X/S}}{1 + b \cdot SRT} \right) \quad (7)$$

167 where  $P_X$  is the biomass production rate (gVSS/day) and  $Q_{O_2\text{biomass}}$  is the oxygen  
 168 consumption rate by the biomass (gO<sub>2</sub>/day).  $P_X$  represents the mass flow rate of biomass  
 169 leaving the system, which at steady state coincides with the biomass production rate in  
 170 the system, while  $Q_{O_2\text{biomass}}$  represents the rate at which biomass consumes oxygen in  
 171 the reactor. Equations (6) and (7) show that, for a given influent composition, the  
 172 biomass produced and the oxygen consumption per unit volume of treated wastewater  
 173 depend only on the SRT.

174 If activated sludge processes are operated in a range of SRT and HRT and data on  
 175 substrate and biomass concentration in the biological reactor are collected, the  
 176 parameters  $Y_{X/S}$  and  $b$ , which determine the production of biomass and the oxygen  
 177 consumption in the reactor, can be determined by the following linearised equation:

178 
$$\frac{SRT(S_0 - S)}{X \cdot HRT} = \frac{1}{Y_{X/S}} + \frac{b}{Y_{X/S}} SRT \quad (8)$$

179 Equation (8) shows that by plotting the variable  $\frac{SRT(S_0 - S)}{X \cdot HRT}$  vs the SRT, we should be  
 180 able to calculate  $Y_{X/S}$  and  $b$  from the slope and intercept of the regression line.

181 The design of the secondary settling tank is affected by the settling rate of the sludge,  
 182 which is inversely proportional to the biomass concentration in the biological reactor,  
 183 e.g. an exponential decay equation is often used:

184 
$$u_c \left( \frac{m}{h} \right) = \alpha e^{-\beta X} \quad (9)$$

185 where  $u_c$  is the settling rate,  $\alpha$  and  $\beta$  are parameters. Equation (9) shows that the higher  
 186 the biomass concentration in the reactor, the lower the settling velocity and therefore the  
 187 larger the area required for the settling tank.

188 In summary this background theory shows that, for a wastewater of given flow rate and  
189 composition and for given kinetic parameters:

- 190 - Lower reactor volumes are achieved by decreasing the HRT and, as a  
191 consequence, by increasing the OLR;
- 192 - Lower reactor volumes give, for a fixed SRT, higher biomass concentrations;
- 193 - Higher biomass concentration can have a negative effect on the settling rate and  
194 therefore on the design of the secondary settling tank;
- 195 - For a fixed HRT, the biomass concentration depends on the SRT, and can be  
196 decreased by decreasing the SRT, as long as the SRT is long enough for the  
197 desired COD removal;
- 198 - Lower SRT gives lower oxygen consumption and higher biomass production.

199 In conclusion, the analysis of the background theory shows that, in theory, for a given  
200 flow rate and composition of the influent wastewater, the appropriate choice of the  
201 parameters HRT and SRT can give the optimum combination of high substrate removal,  
202 low reactor volume, low biomass concentration, low oxygen consumption and high  
203 biomass production.

204 This paper aims to verify this theory experimentally and to identify the optimum  
205 boundary of the parameters HRT and SRT which minimise the reactor volume and  
206 oxygen consumption. The study was carried out using synthetic wastewaters made of  
207 readily biodegradable substrates. Instead of using a continuous-flow process, our  
208 experimental study used sequencing batch reactors (SBRs). In SBRs, reaction and  
209 settling are carried out in the same tank and the process is operated as a sequence of  
210 phases and cycles, rather than as in continuous flow. However, all the concepts and  
211 definitions used in this section apply to SBRs as well, but it has to be considered that  
212 SBRs have additional design parameters compared to continuous-flow systems, i.e. the  
213 number of cycles and the length of the various phases (Dionisi et al., 2016). In our study

214 the only design parameter, in addition to HRT and SRT, which was changed  
215 significantly in one of the runs is the length of the feed and its effect will be discussed  
216 in the Results and Discussion section.

217

## 218 **3. Methods**

### 219 **3.1 Wastewaters and inoculum**

220 Two wastewaters were used in this study. One wastewater had glucose and one had  
221 ethanol as only carbon source. The concentration of glucose and ethanol was 1 g/l. In  
222 both cases nutrients were added to the wastewater before feeding to the reactors:  
223  $\text{NH}_4\text{Cl}$  (0.8 g/l),  $\text{K}_2\text{HPO}_4$  (3.5 g/l),  $\text{NaH}_2\text{PO}_4$  (2.4 g/l), thiourea (20 mg/l). The inoculum  
224 used in this study was a soil from Craibstone farm in Aberdeen (0.1 gVSS/g soil). The  
225 soil was homogenised and sieved (150  $\mu\text{m}$  size) and then stored in plastic containers  
226 at room temperature before inoculation.

### 227 **3.2 Reactor set-up**

228 The reactors used were glass containers with a working volume of 1L. VELP SP 311  
229 peristaltic pumps (Italy) were used to fill the reactors during fill phases and empty the  
230 reactors during effluent withdrawal phases. A Stuart CD162 magnetic stirrer (UK) and  
231 magnetic stirrer bars were used to ensure mixing in the reactor. Oxygen was supplied  
232 to the well-mixed reactors via fine bubble air diffusers from an Interpet Airvolution AV  
233 Air Pump (UK). Throughout these experiments, the dissolved oxygen concentration  
234 levels in the reactors were always kept high ( $> 2 \text{ mg O}_2/\text{l}$ ) and therefore there was no  
235 oxygen limitation. The length of each treatment phase during a cycle was controlled  
236 using a programmable 20 – 250 V Energenie Four Socket Power Management System  
237 (UK).

### 238 **3.3 Experimental design and SBR operation**

239 A total of twenty SBR runs were carried out, eleven with glucose and nine with  
240 ethanol, with different values of HRT, SRT and OLR. The summary of the operating  
241 parameters of the various runs is reported in Tables 1 and 2 (where VER=volumetric

242 exchange ratio=volume of feed per cycle/reactor volume). The runs were carried out at  
243 room temperature, the temperature in the reactors was measured and was in all cases  
244 in the range 20-22 °C. In all the runs except 1G, 6G, 1E, 5E, the Effluent Withdrawal  
245 phase followed the Settle phase and was used to remove the clarified effluent  
246 supernatant. In runs 1G, 6G, 1E and 5E the SRT and the HRT coincided, therefore the  
247 volume of sludge removed needed to coincide with the volume fed every cycle.  
248 Therefore, in these runs the Effluent Withdrawal phase was set immediately before  
249 the Settle phase and removed the completely mixed sludge, with no removal of the  
250 clarified effluent.

251 The fill and react phase were aerated. The main design parameters were the HRT and  
252 SRT. The HRT was controlled by changing the overall daily flow-rate into the reactors.

253 Changes in the HRT resulted in changes to the VER, because  $VER = \frac{1}{No\ cycles \cdot HRT}$ ,

254 where *No cycles* is the number of cycles per day. *No cycles* was set to 4 for all the runs  
255 except runs 10G and 11G, where it was set to 6 in order to keep the VER below its  
256 maximum value of 100%. Therefore, the length of the cycle was 360 mins for all the  
257 runs except runs 10G and 11G, where it was 240 mins. The SRT in each run was  
258 controlled by changing the sludge withdrawal rate ( $Q_w$ ) and by measuring the solid  
259 losses with the effluent. In all runs except 1G, 6G, 1E, 5E the sludge withdrawal was  
260 done manually once per day from the mixed reactor at the end of the reaction phase.  
261 In runs 1G, 6G, 1E, 5E (SRT=HRT) the sludge withdrawal was done using the Effluent  
262 Withdrawal pump, as described above. The average SRT was calculated at the end of  
263 each run from the steady-state concentrations of solids in the well-mixed reactor and  
264 in the effluent according to equation (2), with  $X_R=X$ . The length of the Fill and Effluent  
265 Withdrawal phases was set to be as short as possible and was limited by the maximum

266 flow rates of the available pumps. In some runs, the length of these phases was longer  
 267 than in other runs due to the availability of pumps with lower maximum flow rate.

268 **Table 1.** Operating parameters for the SBRs treating the glucose wastewater.

Run	HRT (day)	VER (%)	OLR (g COD/l.day)	Q <sub>w</sub> (ml/day)	Aver. SRT (day)	Length of the Phases in each cycle (min)			
						Fill	React	Settle	Effluent Withdr.
1G	4	6.25	0.27	250	4	2	298	58	2
2G	4	6.25	0.27	90	8.7	2	298	58	2
3G	4	6.25	0.27	35	16.3	2	298	58	2
4G	4	6.25	0.27	18	27.3	2	298	58	2
5G	4	6.25	0.27	0	65.3	2	298	58	2
6G	1	25	1.07	1000	1	5	295	55	5
7G	1	25	1.07	350	1.7	5	295	55	5
8G	1	25	1.07	0	37	5	295	55	5
9G	0.5	50	2.14	100	2.6	10	285	55	10
10G	0.25	66.7	4.28	70	3.1	10	180	40	10
11G	0.25	66.7	4.28	0	2.9	10	180	40	10

269

270 **Table 2.** Operating parameters for the SBRs treating the ethanol wastewater.

Run	HRT (day)	VER (%)	OLR (gCOD/l.day)	Q <sub>w</sub> (ml/day)	Aver. SRT (day)	Length of the Phases in each cycle (min)			
						Fill	React	Settle	Effluent Withdr.
1E	4	6.25	0.52	250	4	9	291	51	9
2E	4	6.25	0.52	90	8.2	2	298	58	2
3E	4	6.25	0.52	18	20.9	2	298	58	2
4E	4	6.25	0.52	0	70.8	2	298	58	2
5E	1	25	2.07	1000	1	5	295	55	5
6E	1	25	2.07	360	1.7	5	295	55	5
7E	1	25	2.07	0	5.1	35	265	25	35
8E	1	25	2.07	0	9.4	5	295	55	5
9E	0.5	50	4.14	60	4.9	10	315	25	10

271

272

273 At the start-up, 5 g of the well-sieved soil was mixed with 1 L of wastewater feed. The  
274 cycle was initiated with the settle phase, followed by effluent withdrawal. Then the  
275 first feed was introduced and reactor operation continued according to the  
276 programmed cycle pattern. The length of each run was at least 2 times the average  
277 SRT for the run, with a minimum of 25 days, and, in any cases, each run was operated  
278 until the substrate and biomass concentration and the SRT had reached steady state.  
279 At the end of each run, the reactor was cleaned and a new run was started with a fresh  
280 inoculum. Sampling was done three times per week. Biomass concentration and  
281 substrate concentration in the effluent were measured by sampling the reactors at the  
282 end of the reaction phase, while biomass concentration in the effluent was measured  
283 by sampling the collected effluents from the reactors.

#### 284 **3.4 Analytical methods**

285 Biomass concentration was measured as volatile suspended solids (VSS) in accordance  
286 with Standard Methods (APHA, 1998), using a Whatman 1822 – 047 Grade GF/C glass  
287 fibre filter paper of 1.2  $\mu\text{m}$  pore size. Ethanol concentration using gas chromatography  
288 (GC) using a Thermo Scientific Trace 1300 GC coupled to a Flame Ionisation Detector  
289 (FID). The GC column used was a TraceGold TG-WaxMS B GC column (30 m length).  
290 Glucose concentration was measured using the anthrone method. Prior to the glucose  
291 and ethanol analyses, samples were filtered through a Millet syringe filter of 0.45  $\mu\text{m}$   
292 pore size. Soluble COD in the effluent was also measured, after filtration, using COD  
293 cell test kits (Merck).

#### 294 **3.5 Data analysis**

295 The biomass produced per unit volume of influent wastewater was calculated in each

296 run from the steady-state values of the biomass concentration (X), HRT and SRT  
297 according to equation (10):

$$298 \quad \text{Biomass produced} \left( \frac{\text{g biomass}}{\text{l influent wastewater}} \right) = \frac{\text{HRT} \cdot X}{\text{SRT}} \quad (10)$$

299 The oxygen consumption by the microorganisms was calculated in each run using the  
300 experimental data on biomass produced, influent ( $S_0$ ) and effluent (S) COD  
301 concentrations and using the COD balance, according to equation (11):

$$302 \quad \text{Oxygen consumed} \left( \frac{\text{g oxygen}}{\text{l influent wastewater}} \right) = (S_0 - S) - \frac{\text{HRT} \cdot X}{\text{SRT}} 1.42 \quad (11)$$

303 where the factor 1.42 is the COD conversion factor for biomass, assuming its empirical  
304 formula is  $C_5H_7O_2N$ .

305 The fraction of the removed COD which was converted to biomass was calculated  
306 according to equation (12):

$$307 \quad \text{Fraction of removed COD converted to biomass} = \frac{1.42 \cdot \text{HRT} \cdot X}{\text{SRT} \cdot (S_0 - S)} \quad (12)$$

308 The fraction of the removed COD which was oxidised was calculated from the COD  
309 balance as:

$$310 \quad \begin{aligned} &\text{Fraction of removed COD which was oxidised} = \\ &= 1 - \text{Fraction of removed COD converted to biomass} \end{aligned} \quad (13)$$

311 The kinetic parameters  $Y_{X/S}$  and  $b$  were calculated by linearising the experimental data  
312 according to equation (8) in Section 2.

313

## 314 **4. Results and Discussion**

### 315 **4.1. Minimum SRT for substrate removal**

316 Since the SRT is the only (for continuous-flow systems) or the main (for SBR systems)  
317 parameter that determines the effluent substrate concentration, the first step was to  
318 determine how the glucose and ethanol removal were affected by the SRT (Figure 1).  
319 For both substrates the removal was virtually complete at high SRT and incomplete or  
320 very low at low SRT. The minimum SRT for high removal efficiency (assumed to be  
321 >90%) was in the range 2.5-3.0 days for glucose and 1.7 days for ethanol. For glucose it  
322 can be observed that the removal was complete in Run 9G, operated at an SRT of 2.6  
323 days, while it was incomplete in run 11G, which had an average SRT of 2.9 days. These  
324 two values of the SRT are very similar and indicate that the performance of the process  
325 can be quite unstable if the SRT is close to its lowest limit for complete substrate  
326 removal. For ethanol, substrate removal was incomplete in run 7E, where the SRT was  
327 higher than in runs where complete or almost complete removal was observed (Runs  
328 9E, 4E, 6E). The likely explanation for this behaviour is that in Run 7E the feed length  
329 was the longest among all the investigated runs. Long feed means lower average  
330 substrate concentration during the cycle and therefore lower average substrate  
331 removal rate, for the same value of the SRT (Dionisi et al., 2016).

332 The determination of the minimum SRT that is required for substrate removal is  
333 important because, as discussed in Section 2, the conditions of minimum reactor  
334 volume and minimum oxygen consumption are expected to be found at the lowest  
335 SRT. Considering literature studies where aerobic wastewater treatment was operated  
336 at low SRT, the minimum SRT which was successfully applied for the removal of  
337 organic carbon was 0.6 day (Bloor et al., 1995). That study was carried out on brewery

338 wastewater at an unspecified temperature and achieved the highest reported OLR for  
339 aerobic processes, 52 kg COD/m<sup>3</sup>.day, due to the very low SRT and the use of the jet  
340 loop reactor. Jimenez et al. (2015) obtained a COD removal of approximately 80% with  
341 SRT of 2 days. Ge et al. (2013, 2017) successfully operated aerobic treatment at SRT  
342 values in the range 1.5-3 day at 20-22 °C. For a synthetic glucose-based wastewater at  
343 thermophilic (58 °C) temperatures, the efficiency of COD removal was found to  
344 decrease for SRT lower than 2-3 days (Surucu et al., 1976), in agreement with the  
345 present study. In summary, while there is little literature study for the minimum SRT  
346 for ethanol as only carbon source, overall our data on the effect of SRT on process  
347 performance are in agreement with other literature studies and confirm the possibility  
348 of achieving high efficiencies of COD removal even at low values of the SRT. Since the  
349 minimum SRT has implications for the minimum HRT and maximum OLR and for the  
350 minimum oxygen consumption, further study will need to be dedicated to determine  
351 the minimum SRT for more complex wastewaters, which include slowly biodegradable  
352 substrates, and for nitrification/denitrification processes, when nitrogen removal is  
353 required.

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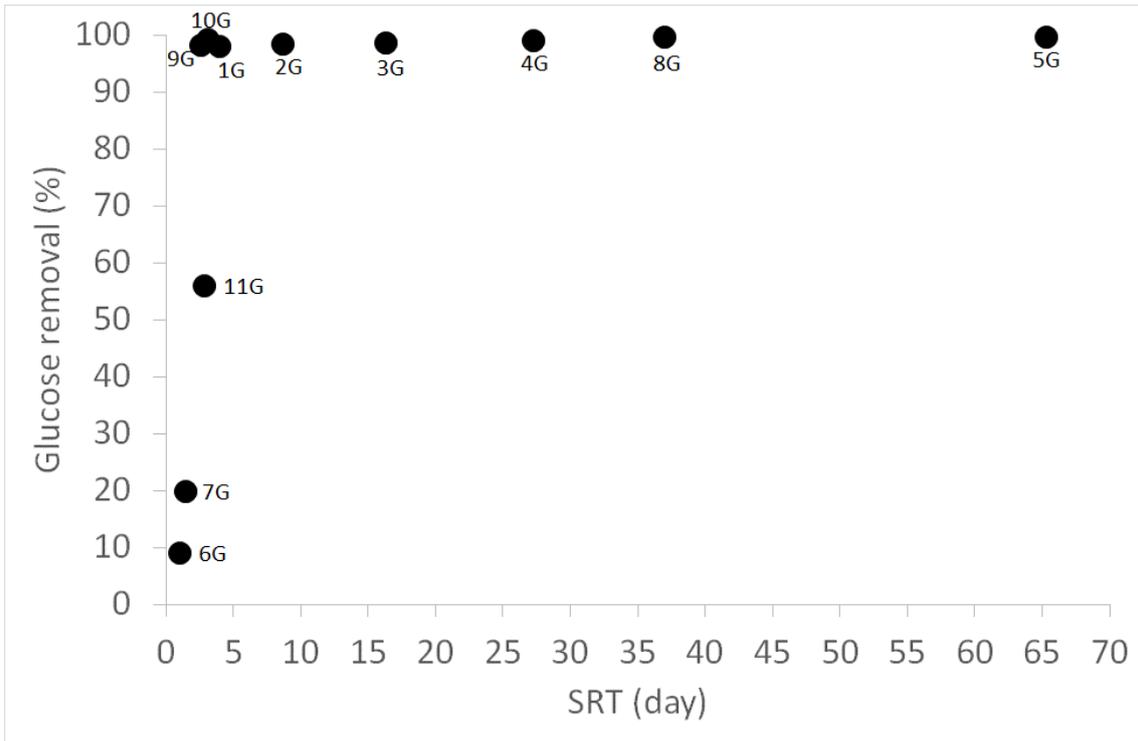
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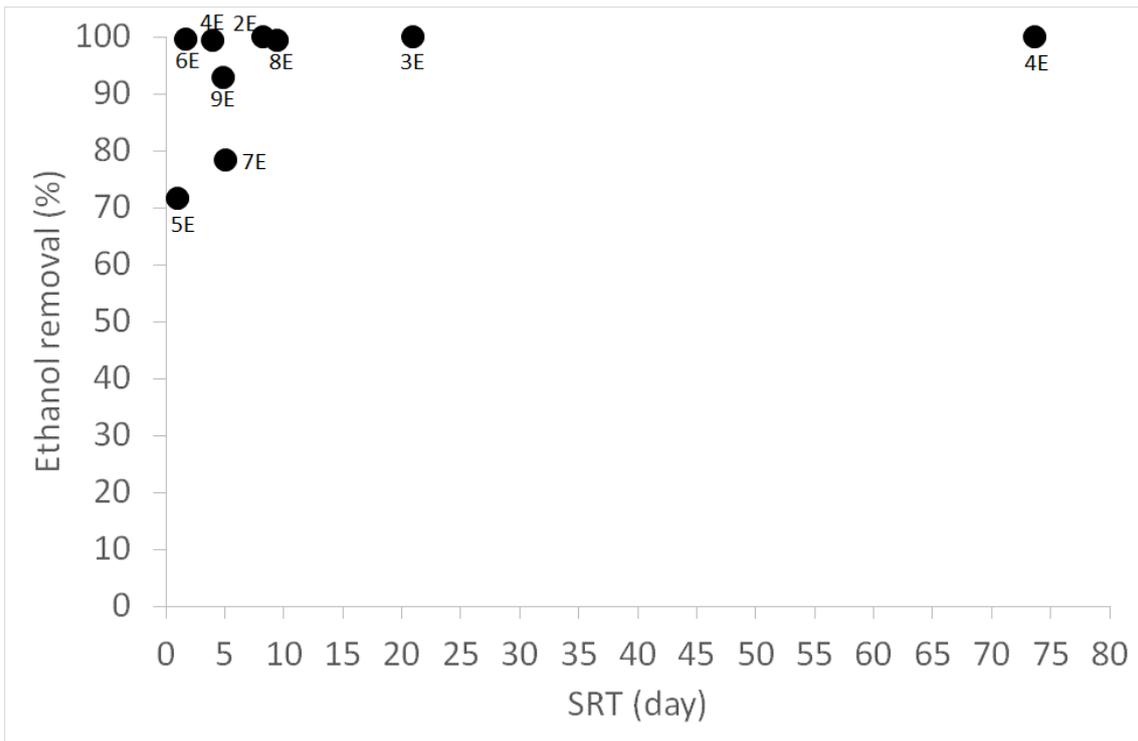
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362 **Figure 1.** Effect of the SRT on the glucose (top) and ethanol (bottom) removal.

363

#### 364 4.2. Maximisation of the OLR

365 Figure 2 shows the effect of the HRT (or of the OLR, which is inversely proportional to  
366 the HRT) on the biomass concentration in the reactor. For a fixed HRT (or OLR), the  
367 biomass concentration is a function of the SRT, as expected, as shown, in the runs at  
368 0.27 g COD/l.day for glucose and at 0.52 g COD/l.day for ethanol. As the OLR is  
369 increased (i.e. the HRT is decreased), the biomass concentration was kept within  
370 acceptable levels by decreasing the SRT. For example, in the glucose reactors the  
371 biomass concentration was very high, 6.9 g VSS/l, in Run 8G (OLR equal to 1.07 g  
372 COD/l.day and SRT 37 days) and the OLR could not have been increased further at the  
373 same SRT, otherwise the biomass concentration would have been too high and the  
374 settling rate would have been compromised. Therefore the runs at higher OLR (Runs  
375 9G, 10G, 11G at OLR of 2.14 and 4.28 g COD/l.day) were carried out at lower SRT, in  
376 the range 2.6-3.1 days. This allowed obtaining lower biomass concentrations at high  
377 OLR than at low OLR, confirming what was expected according to the background  
378 theory in Section 2. The same effect was observed for ethanol. For example, thanks to  
379 their lower SRT, Runs 8E and 9E had lower biomass concentration in the reactor than  
380 Run 4E, in spite of their higher OLR.

381 The operation at high OLR can only be considered successful if the high OLR does not  
382 impact negatively on the settleability of the sludge, which in this study was measured  
383 by the biomass concentration in the effluent collected after the settling phase (Figure  
384 3). In Figure 3, runs 1G, 6G, 1E, 5E are not reported because in those runs the SRT was  
385 set equal to the HRT and the effluent was collected from the completely mixed  
386 reactor, with no effluent collection after the settling phase. For the glucose runs, the  
387 biomass in the effluent was in the range 100-250 mg VSS/l for all the runs except Run

388 11G. The high solid losses in the effluent in Run 11G can be explained considering that  
389 in this run a high OLR was applied and no sludge withdrawal. In the absence or with  
390 low solid losses in the effluent, this would have caused a very high biomass  
391 concentration in the reactor with consequent very low settling velocity. Therefore, the  
392 high solid losses in the effluent were the reaction of the system to the high OLR with  
393 no sludge withdrawal and indicated that the process cannot be operated at high OLR  
394 without control of the SRT. In summary, as far as the maximisation of the OLR is  
395 concerned, the most successful run for the glucose reactor was Run 10G, where the  
396 high OLR of 4.28 g COD/l.day was maintained with complete substrate removal and  
397 with solid losses in the effluent which were similar to the other runs. For the ethanol  
398 runs, the solid losses in the effluent were always in the range 150-300 mg/l, indicating  
399 that the highest OLR could be maintained without a negative impact on this variable.  
400 Interestingly, the highest solid losses with the effluent were observed for Run 7E,  
401 where the feed length was the longest, therefore indicating that the long feed length  
402 has a negative effect on the settling properties. Indeed runs 6E, 7E, 8E were operated  
403 at the same OLR and HRT but the length of the Fill phase was considerably longer in  
404 run 7E (35 mins vs 5 mins in runs 6E and 8E). In SBRs, the shorter the feed length, the  
405 higher the substrate gradients in the system, and high substrate gradients are known  
406 to favour the development of well settling sludge (Dionisi et al., 2006a; Martin et al.,  
407 2003). For the ethanol runs it can be concluded that the run that gave the highest OLR  
408 with an acceptable performance was Run 9E, with a OLR of 4.14 g COD/l.day, over 90%  
409 substrate removal and acceptable solid losses in the effluent.

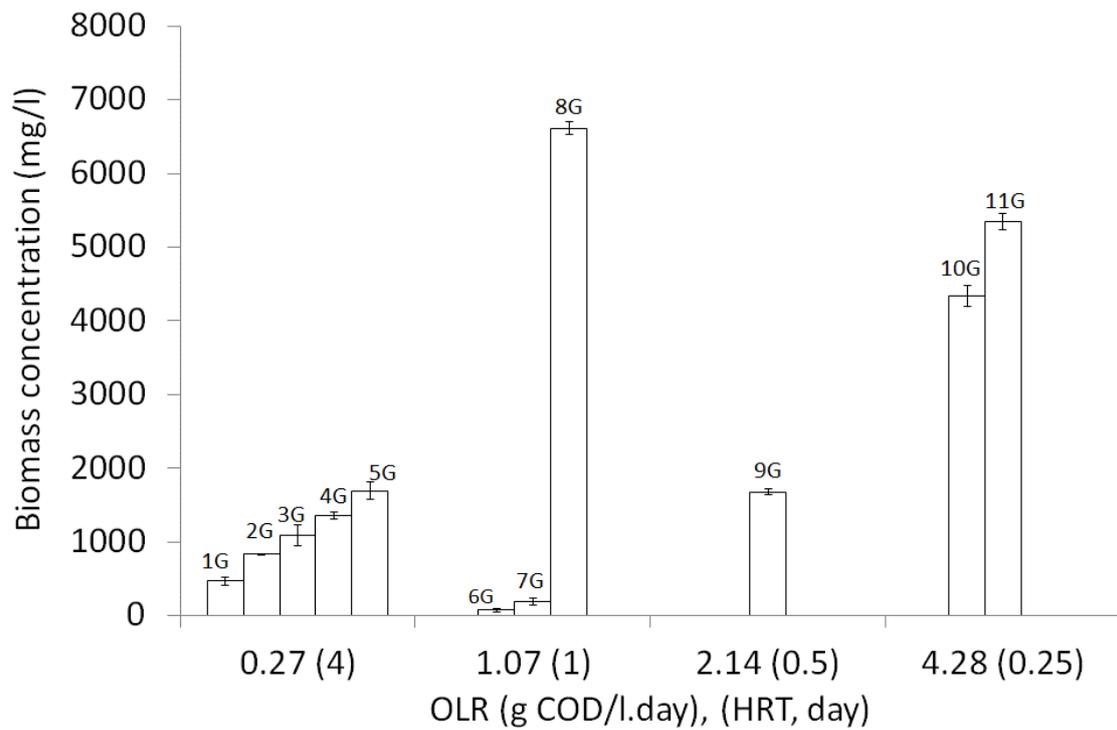
410 The maximum values of the OLR determined in this study, 4.28 and 4.14 g COD/l.day,  
411 are among the highest reported for aerobic suspended-growth conventional activated

412 sludge processes (Table 3). In Table 3 we have not considered non-conventional  
413 processes, e.g. the air bubble or the jet loop reactor discussed in the Introduction,  
414 membrane reactors or granular sludge. However, it is important to observe that the  
415 high OLRs obtained in this study are in the range of values reported for membrane or  
416 granular reactors, e.g. Trussel et al. (2006) reported operation of membrane  
417 bioreactors in the OLR range 2.2-8.2 g COD/l.day, which are among the highest  
418 reported for MBRs, and Liu et al. (2005) operated granular-sludge reactors with OLRs  
419 of up to 4.0 g COD/l.day, even though granulation allowed the achievement of OLR as  
420 high as 15 g COD/l.day (Moy et al., 2002).

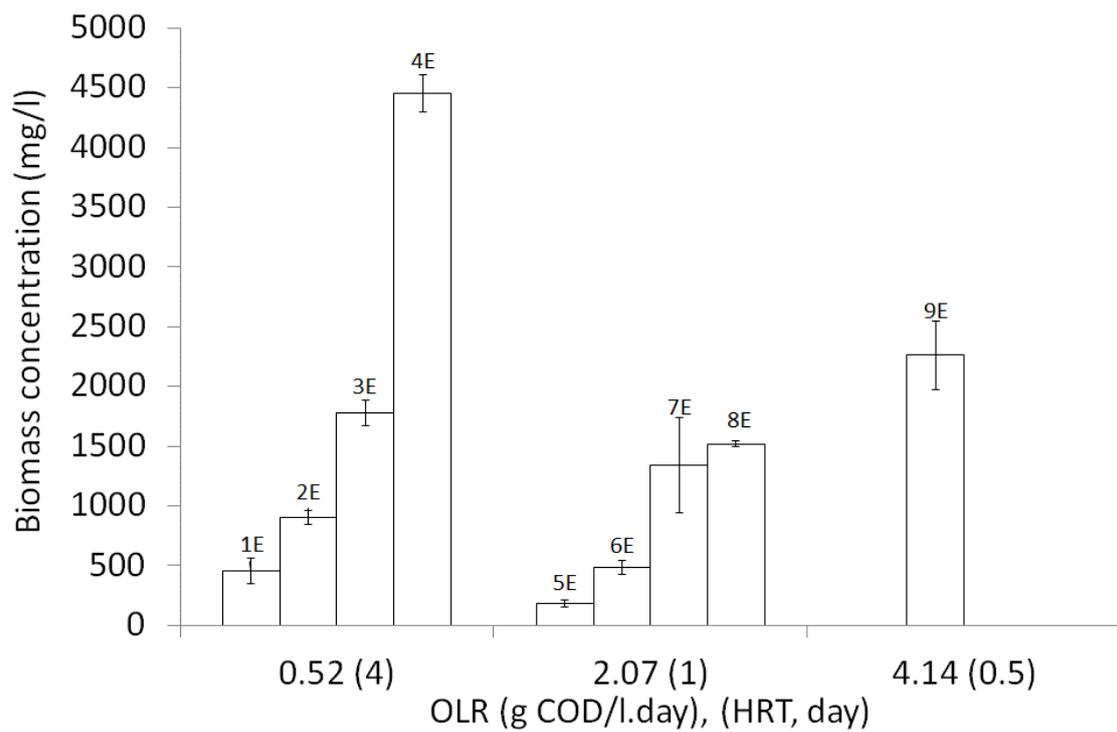
421 In summary, our experimental study has showed that the simultaneous optimisation of  
422 the HRT and SRT allows the operation of conventional suspended-growth processes at  
423 very high OLR, with consequent minimisation of the reactor volume and plant  
424 footprint.

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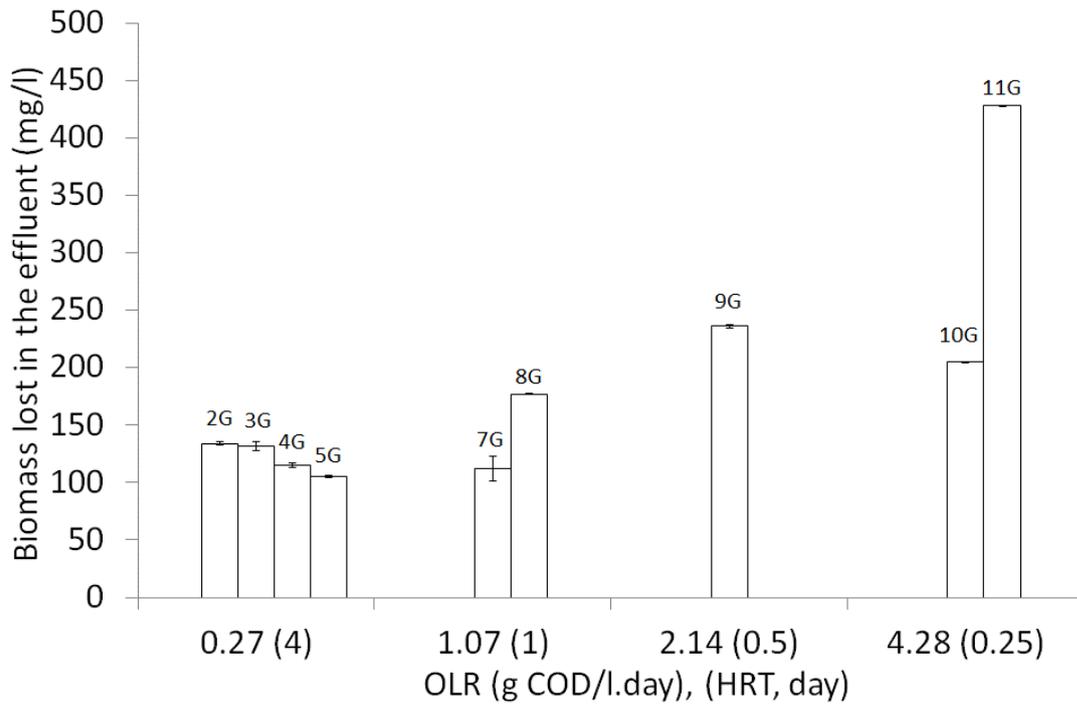
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429 **Figure 2.** Biomass concentration at the end of the reaction phase for the glucose (up)  
 430 and ethanol (bottom) reactors.

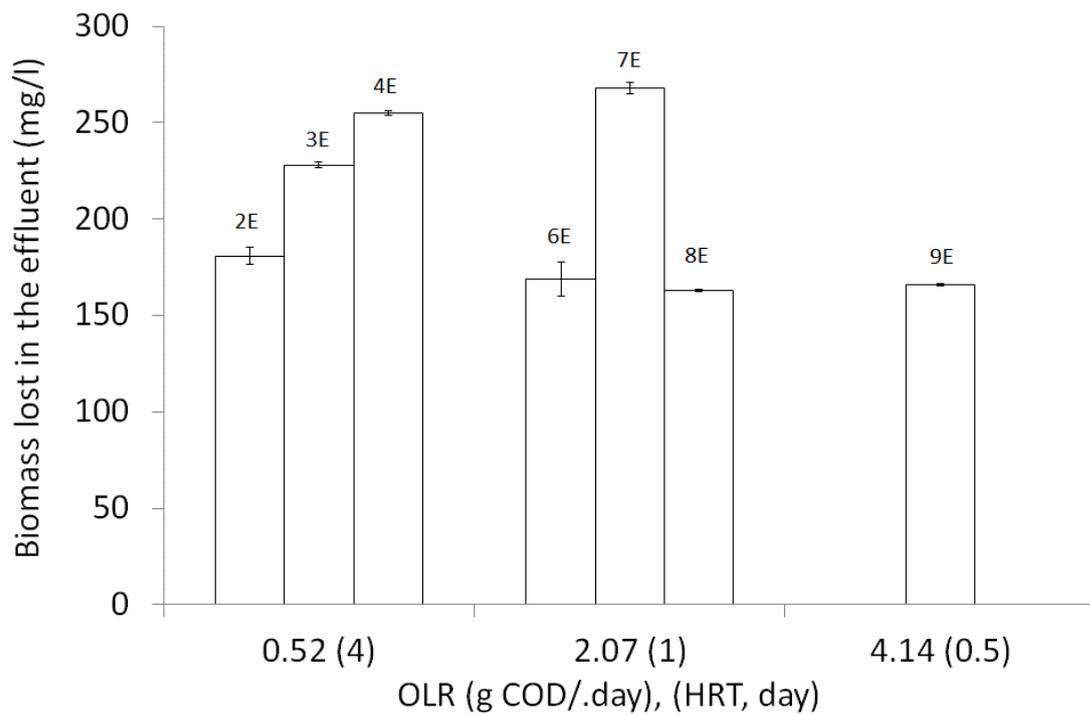
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436 **Figure 3.** Biomass concentration in the effluent for the glucose (up) and ethanol  
 437 (bottom) reactors.

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445 **Table 3.** Aerobic studies carried out at high OLR with conventional suspended-growth  
 446 activated sludge processes.

Reference	Wastewater	HRT (day)	SRT (day)	OLR (g COD/l.day)
Kanimozhi et al. (2014)	Anaerobically digested distillery	1.0	N.R.	3.6
Ge et al. (2013)	Slaughterhouse	0.5	2	5.8
Rodríguez et al. (2013)	Animal food factory	0.75	30	4.55
Yoong et al. (2000)	Phenol	0.42	4	3.12
This study (glucose)	Glucose	0.25	3.1	4.28
This study (ethanol)	Ethanol	0.5	4.9	4.14

447

### 448 **4.3. Minimisation of oxygen consumption**

449 In addition to the OLR, the optimum design of biological processes requires the  
 450 minimisation of the oxygen consumption and the maximisation of the produced  
 451 biomass, assuming that the produced biomass is used in anaerobic digesters for energy  
 452 generation. Figure 4 shows the oxygen consumption and the produced biomass for the  
 453 glucose and ethanol reactors. It is expected that the biomass produced and oxygen  
 454 consumed (per unit volume of influent wastewater) only depend on the SRT (equations  
 455 (6) and (7) in Section 2). However, both the glucose and ethanol runs indicate that, in  
 456 disagreement with the theory, the OLR also affects the biomass and oxygen  
 457 production. Indeed, for the glucose reactor Runs 1G-5G and 8G give the expected  
 458 trend, while Runs 10G and 9G give lower biomass produced and higher oxygen  
 459 consumption than the other runs, in spite of their lower SRT. Similarly for ethanol,  
 460 Runs 1E-4E gave the expected trend, while Runs 6E, 9E and 8E gave lower biomass  
 461 production (and hence higher oxygen consumption) in spite of having similar SRT as  
 462 the other Runs. In general the results obtained with the two substrates indicate that at  
 463 higher OLR the biomass production decreases for the same SRT, and this causes, from

464 the COD balance, an increase in oxygen consumption. More insight into biomass  
465 production and oxygen consumption is shown in Figure 5, which shows the fraction of  
466 the removed COD which is converted into biomass or oxygen in the various runs. The  
467 trend is the same as reported in Figure 4, however Figure 5 highlights an important  
468 difference between glucose and ethanol. For glucose, the minimum value of the  
469 fraction of oxidised COD is 36% (Run 1G), while for ethanol it is 69% (Run 1E) and in  
470 general the fraction of oxidised COD, i.e. the oxygen consumption by the  
471 microorganisms, is significantly larger for glucose than for ethanol. In general, the  
472 results of this study indicate that, at least for the wastewaters considered here, the  
473 operating parameters that give the maximum organic load are not the same that give  
474 the minimum oxygen consumption. If minimising oxygen consumption is the priority,  
475 the operating conditions of Runs 1G and 1E, low OLR and low SRT, are to be preferred  
476 while if the minimisation of reactor volume is the priority, the conditions of Runs 10G  
477 and 9E, high OLR and low SRT, have to be chosen.

478 The obtained data were analysed to calculate the kinetic parameters  $Y_{X/S}$  and  $b$  (Figure  
479 6). For the glucose runs, Runs 1G-5G and 8G were considered, while Runs 9G and 10G  
480 were excluded, because of their deviation from the theory. For the ethanol runs, two  
481 plots were generated, one for the runs at lower OLR and one for the runs at higher  
482 OLR. For glucose, the obtained values of the parameters were  $Y_{X/S} = 0.60$  g biomass/g  
483 COD and  $b = 0.08$  day<sup>-1</sup>. For the ethanol runs we obtained, at higher OLR,  $Y_{X/S} = 0.18$  g  
484 biomass/g COD,  $b = 0.13$  day<sup>-1</sup>, and, at low OLR,  $Y_{X/S} = 0.23$  g biomass/g COD and  $b =$   
485 0.01 day<sup>-1</sup>.

486 The lowest oxygen consumption found in this study, 0.36 kg O<sub>2</sub>/kg COD removed, is  
487 among the lowest reported in the literature for aerobic processes. Surucu et al. (1976)

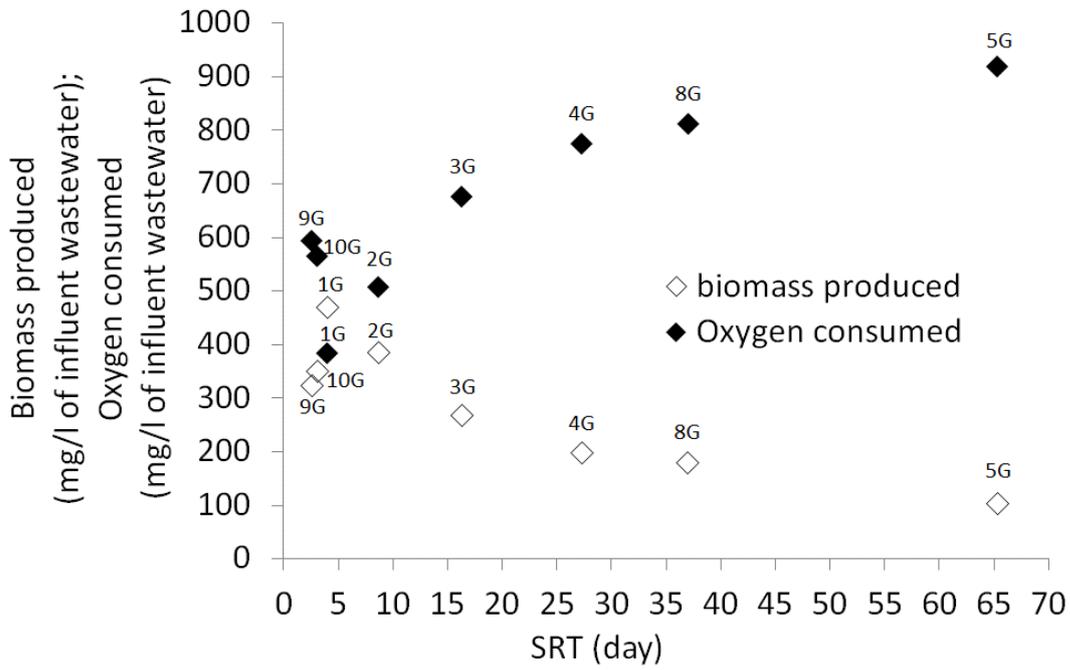
488 reported an oxygen consumption of approximately 0.65 kg O<sub>2</sub>/kg COD removed at a  
489 SRT of 2 day. Ge et al. (2013, 2017) obtained an oxygen consumption of 0.15-0.3 kg  
490 O<sub>2</sub>/kg removed COD at SRT values of 2-3 day and Jimenez et al. (2015) reported oxygen  
491 consumptions in the range 0.2-0.5 kg O<sub>2</sub>/kg COD in the SRT range 0.1-2 days. When  
492 studies are carried out at larger SRT, much larger oxygen consumptions are observed,  
493 e.g. Ouyang and Junxin (2009) observed over 0.70 kg O<sub>2</sub> consumed/kg COD for SRT of  
494 10 day.

495 The decrease in observed yield which we observed at higher OLR has important  
496 consequences for the design of biological wastewater treatment processes. From the  
497 point of view of maximising the OLR, it can be considered an advantage, because it  
498 means that the biomass concentration does not increase linearly as the OLR is  
499 increased, for a fixed SRT. This means, in turn, that higher OLR values are possible than  
500 what is possible to estimate based on the biomass concentrations obtained at low OLR  
501 values. However, from the point of view of the simultaneous minimisation of reactor  
502 volume and oxygen consumption, the decrease in observed yield as the OLR increases  
503 is a disadvantage. Indeed, our study shows that the runs with the highest OLR and  
504 lowest SRT are not the ones which give the lowest oxygen consumption. This is not in  
505 agreement with the theory reported in Section 2, however, a decrease in observed  
506 yield at higher OLR has already been reported by Dionisi et al. (2006b). Our kinetic  
507 analysis for the ethanol runs shows that the reason for the lower biomass production  
508 and higher oxygen consumption observed at high OLR is mainly the fact that at high  
509 OLR the microbial kinetics is described by a larger value of the endogenous metabolism  
510 coefficient  $b$ . Indeed, for ethanol the parameter  $b$  was 0.13 day<sup>-1</sup> at higher OLR and  
511 0.01 day<sup>-1</sup> at lower OLR, while the parameter  $Y_{X/S}$  was only slightly different (0.18 vs

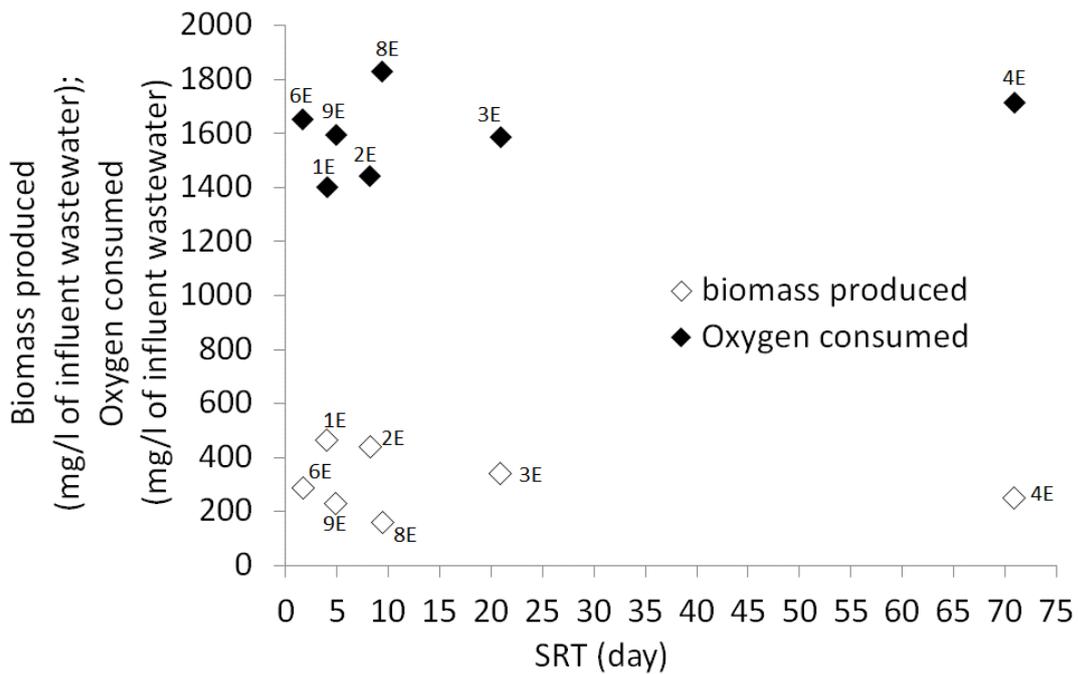
512 0.23 g biomass/ g COD) at higher and lower OLR. It remains to be investigated whether  
513 this effect of the OLR on the rate of endogenous metabolism is specific for the  
514 wastewaters considered here or is more general. If it is general, then conventional  
515 models for biological wastewater treatment processes will need to be modified, e.g. by  
516 using different values of the endogenous metabolism parameter at different values of  
517 the OLR. The kinetic analysis also shows that the reason for the higher biomass  
518 production and lower oxygen consumption for glucose than for ethanol is in the higher  
519 growth yield ( $Y_{X/S}=0.60$  g biomass/g COD for glucose,  $Y_{X/S}=0.18-0.23$  g biomass/g COD  
520 for ethanol).

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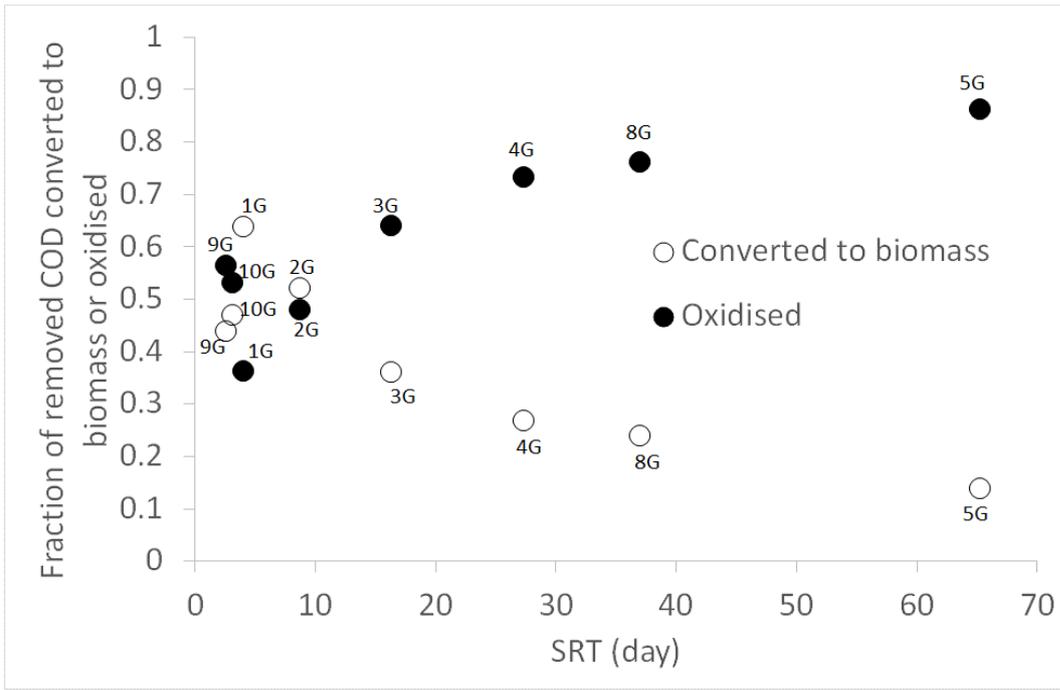
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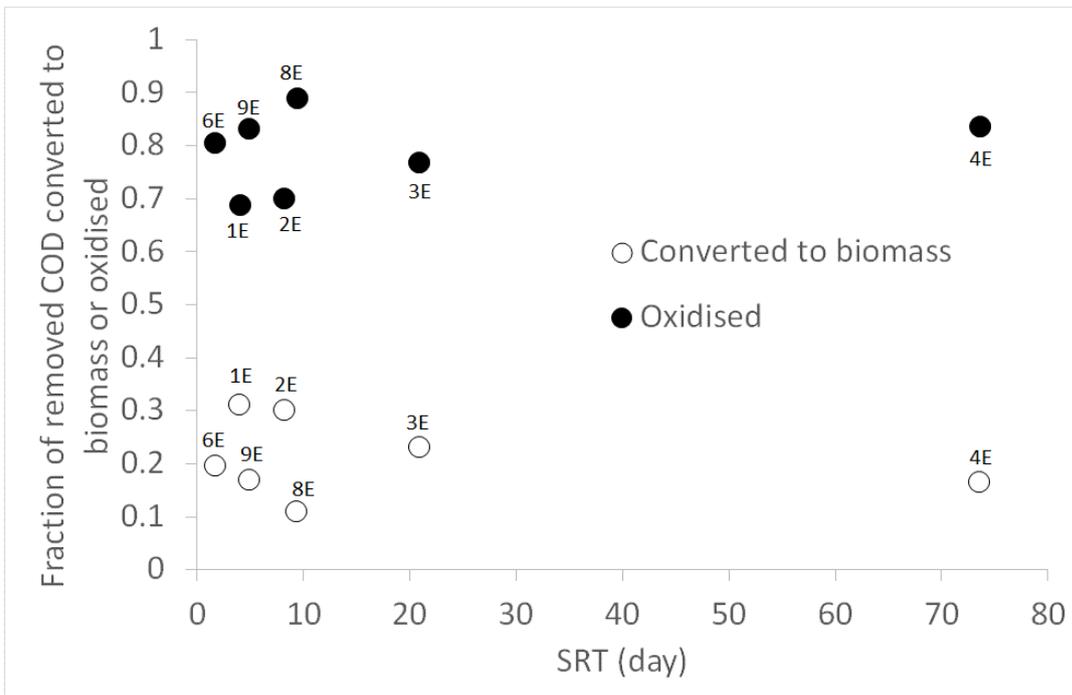
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525 **Figure 4.** Biomass produced and oxygen consumed for the glucose (top) and ethanol  
526 runs (bottom).

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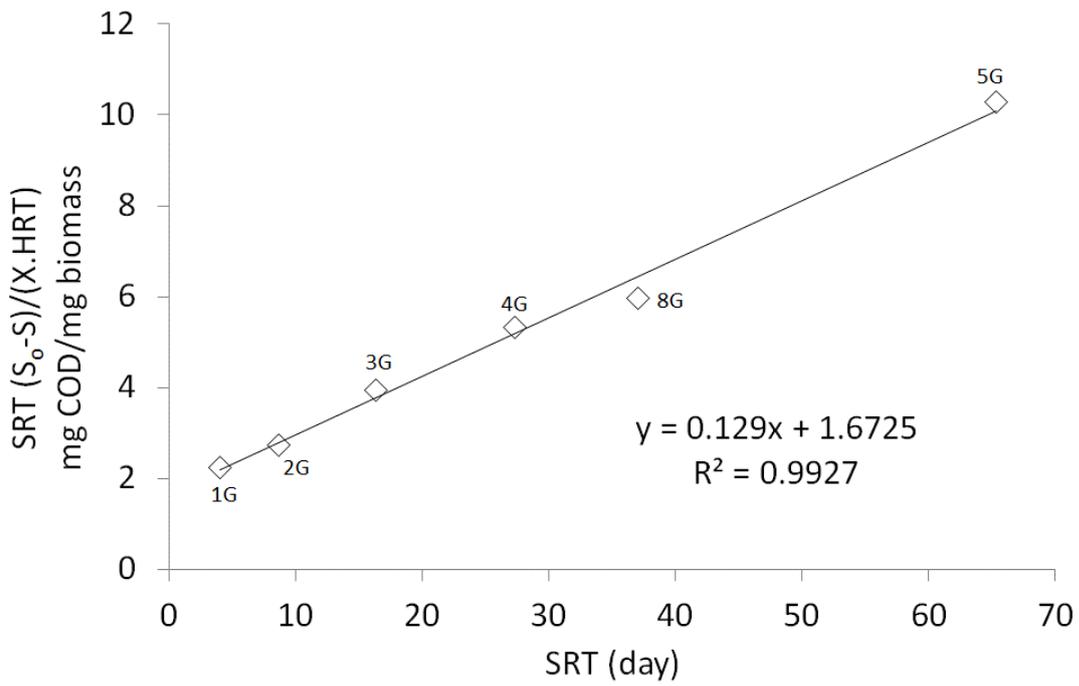


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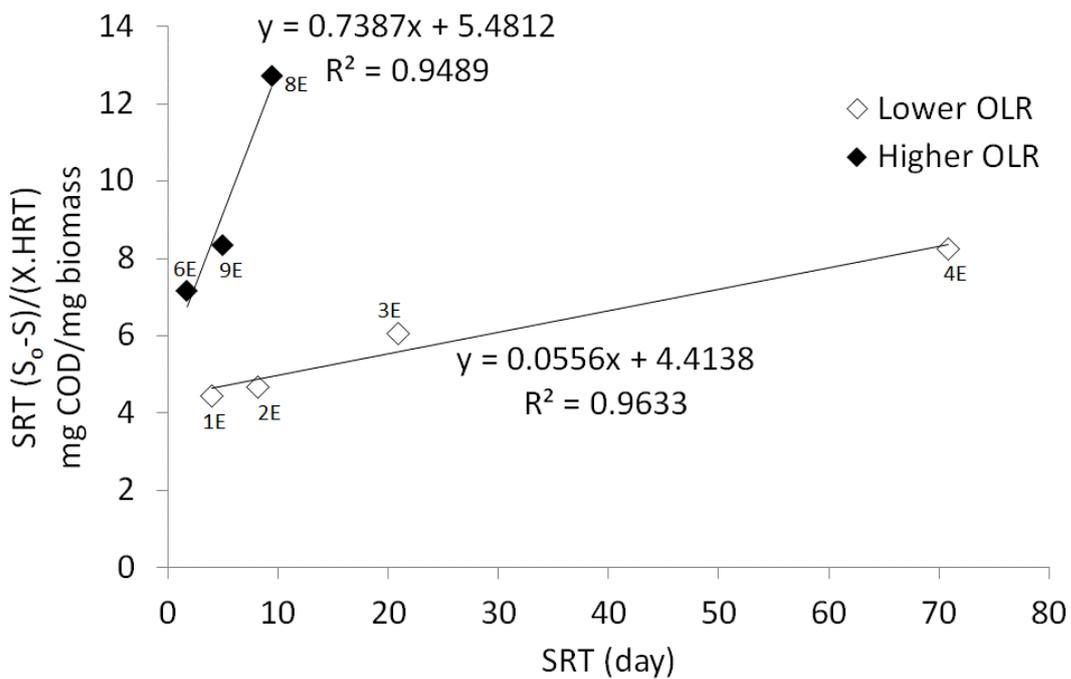


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530 **Figure 5.** Distribution of the removed COD between oxidised and converted to biomass  
 531 for the glucose (top) and ethanol (bottom) runs.  
 532



533



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535 **Figure 6.** Linearisation of the experimental data for the calculation of the kinetic  
 536 parameters  $Y_{X/S}$  and  $b$ . Glucose (top) and ethanol (bottom) runs.

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541 **4. Conclusion**

542 This study has shown that it is possible to operate conventional suspended-growth  
543 aerobic processes at high OLR, up to 4.28 g COD/l.day, by simultaneous optimisation of  
544 the HRT and SRT. The operating conditions which gave the highest OLR, and therefore  
545 the minimum reactor volume, were HRT=0.25 day and SRT=3.1 day for the glucose  
546 wastewater and HRT=0.5 day and SRT=4.9 day for the ethanol wastewater.

547 The values of the HRT and SRT that gave the minimum oxygen consumption were not  
548 the same that gave the highest OLR. The minimum oxygen consumption was obtained  
549 at HRT=SRT=4 day for both glucose and ethanol. The oxygen consumption per unit of  
550 COD removed was higher for ethanol than for glucose. The minimum oxygen  
551 consumption was 0.36 and 0.69 kg O<sub>2</sub>/kg COD removed for glucose and ethanol  
552 respectively.

553 In disagreement with the conventional theory, biomass production and oxygen  
554 consumption per unit of removed substrate were observed to depend on the OLR as  
555 well as on the SRT. Biomass production decreased and oxygen consumption increased  
556 at higher OLR. This behaviour has important consequences for the design of biological  
557 wastewater treatment processes and will need to be investigated further with  
558 wastewaters of different composition.

559 Overall this study has shown the importance of optimising the SRT and HRT to achieve  
560 the optimum performance of the process. Further study is needed for wastewaters of  
561 different and more complex composition and for nitrification/denitrification processes  
562 for nitrogen removal.

563

564 **Acknowledgement**

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566 and appreciated.

567

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