High-pressure jet device for activated sludge reduction: Feasibility of sludge solubilization

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Abstract

The objective of this study was to investigate the feasibility of a high-pressure jet device (HPJD) as an alternative technology to reduce the amount of excess activated sludge in a low-cost and simple manner. The device received concentrated activated sludge from two different ports: a horizontal port that transferred activated sludge with a jet flow via a nozzle and a side port that aspirated activated sludge with air. An investigation into the manner in which activated sludge was supplied indicated that the activated sludge conveyed by horizontal jet flow with aspiration from the elevated side port provided the highest degree of activated sludge reduction in the mixed liquor suspended solid (MLSS). The degree of reduction was 12.2% and the viable microorganisms decreased by 42.8%. The injection ratio R was defined as the amount of activated sludge from the side port to that of the nozzle. We found an optimum value of three with a MLSS reduction of 40%. The eluted constituents from the damaged cells or the intracellular compounds were biodegraded by the intact activated sludge. Estimation on cost and performance for activated sludge reduction, based on the energy and MLSS reduction, showed that the HPJD is twice as cost-effective as conventional technologies and that the throughput is 3.2-7.2 times as high as a high pressure homogenization device.

Keywords: Activated Sludge; Aggregation; Agitation; Bubbles; Cell Disruption; Reduction; Waste-Water Treatment

19 **1. Introduction**

20 Activated sludge systems are important in industrial and municipal wastewater treatment
21 plants (WWTPs) [1]. Activated sludge systems produce excess sludge because of excess growth of
22 microorganisms incorporating organic carbon and nitrogen compounds dissolved in incoming
23 wastewater. The cost of excess sludge treatment accounts for 18–57% of the total operational cost
24 of WWTPs, and this is, therefore, an engineering challenge [2]. This challenge warrants the
25 development of a system to reduce the amount of excess activated sludge.

26 A mechanism of excess activated sludge reduction is generally destruction of bacterial cell
27 walls and membranes by a sludge reduction technology, which releases organic carbon to be
28 mineralized into carbon dioxide by metabolisms of intact microorganisms in a bioreactor. Several
29 technologies, based on chemical, physical and biological methods to reduce excess activated sludge
30 production, have been developed. Ozonation is one of the most broadly used chemical treatment
31 technologies [3-6]. Alternative chemical technologies for excess activated sludge reduction are
32 chlorination [7-10] and alkali-thermal treatment, and these technologies have previously been
33 investigated [11-13]. Milling treatment [14], thermal processing [15], and ultrasonic treatment
34 [16-18] are representative physical treatment technologies for excess activated sludge, which have
35 been intensively investigated due to their high throughput and simplicity. However, the
36 disadvantages of these chemical treatments are the high cost of chemical agents and the cost of
37 electricity for the generation of ultrasonic waves and heat to solubilize microorganism cells [19].
38 On the other hand, anaerobic or aerobic sludge digestion, a representative biological method, is an

option to reduce excess activated sludge. However, biological treatment requires stringent operational conditions, e.g. temperature, solid retention time, volumetric organic loading rate, pH and so on, to sustain stability of activated sludge reduction performance [20-22].

To reduce excess activated sludge in a cost-effective and high-throughput manner, a high-pressure jet device (HPJD) was developed. This device has originally been applied for washing of soil contaminated with insulation oil, resulting in high washing performance of the soil in an extremely short time [23]. This technology potentially broadens the applicability to excess activated sludge reduction. The HPJD consists of a high-pressure pump, which transfers the activated sludge, and pipes of different diameters together with a collision plate at the edge (Figure 1). Two influent ports are used for activated sludge injection in a jet flow manner through a nozzle and the sludge is drawn at the top of the pipe (side port). Jet flow is generated by lateral flow through the nozzle and this enhances the negative pressure in a HPJD so that activated sludge from the side port is taken with air, resulting in intensive mixing in the pipe. The accepted mechanisms of activated sludge solubilization are (1) cavitation, (2) frictional shear and (3) collision. Cavitation is caused by the transport of activated sludge into a zone with a narrower diameter pipe in which the formed fine bubbles collapse, resulting in a significant increase in temperature [24]. Frictional shear likely occurs at the point where the activated sludge that falls vertically from the top port contacts the laterally transferred sludge. The high velocity of the activated sludge expectedly leads to a compression of microorganism cells on the steel plate at the end of the pipe. These multiple effects potentially enhance the solubilization of the microorganism cells leading to excess activated sludge

59 reduction. In addition, the forced sludge aspiration accelerates the throughput capacity of the
60 activated sludge to be treated. A similar device without a side port from which activated sludge is
61 fed has been designed for the enhancement of cavitation for water sterilization [25], pretreatment
62 for anaerobic digestion [26] and activated sludge reduction [27]. However, the application of a
63 HPJD device where multiple factors are at work in addition to cavitation has not been investigated
64 to date. Because of these multiple factors, which are distinct from a high pressure homogenization
65 (HPH) where cavitation is a main factor for cell destruction [27], a HPJD is a promising simple,
66 high-throughput and chemical-free sludge reduction technology. Additionally, it can be installed
67 into the return of a sludge line in an activated sludge system.

68 This study was therefore undertaken to evaluate the effectiveness of a HPJD to reduce the
69 volume of activated sludge from a municipal wastewater treatment plant by batch experiments.
70 Specifically, the manner and degree of activated sludge supply from the two ports were investigated
71 by measuring the magnitude of the reduction effect, the viability of bacterial cells and the
72 availability of treated activated sludge as a substrate after HPJD application.

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74

75 **2. Materials and Methods**

76 **2.1. High-pressure jet device (HPJD)**

77 Activated sludge was injected into the HPJD, *a.k.a.* the DEM[®] (Dojo-Kankyo Process
78 Institute, Yokohama, Japan). The HPJD has two influent lines where activated sludge is supplied as

79 shown in Figure 1. One of the two lines was supplied horizontally and the other vertically. The
80 activated sludge that was added horizontally was transferred using a nozzle and a high pressure
81 pump (HPJ-160, Tsurumi Manufacturing Co., Ltd., Osaka, Japan). Because of waste activated
82 sludge handling issues, two HPJDs with different throughput capacities were made: one was used to
83 investigate the effect of activated sludge on the degree of solubilization and it had a total length of
84 1.5 m, a nozzle diameter of 2.5 mm and a high pressure pump flow rate of 27 L/min, which ensured
85 a maximum pressure of 4.5 MPa, whereas the other had a total length of 1 m, a nozzle diameter of
86 0.7 mm and a high pressure pump flow rate of 10 L/min, ensuring a maximum pressure of 6 MPa.

87 **2.2. Source of activated sludge**

88 The activated sludge used in this study was obtained from a line of concentrated activated
89 sludge from a secondary clarifier in the full-scale municipal WWTP (Kitatama Ichigo Water
90 Reclamation Center, Tokyo, Japan). This WWTP employed a conventional activated sludge system,
91 continuously receiving municipal wastewater with average pH, COD_{Mn}, BOD, NH₄⁺, total
92 phosphorus and suspended solid (SS) concentrations of 7.61 ± 0.29, 100 ± 19 mg/L, 153 ± 38 mg/L,
93 18.3 ± 2.3 mg-N/L, 3.3 ± 0.4 mg-P/L and 153 ± 70 mg/L, respectively. Hydraulic and solid
94 retention times of the aeration tank were controlled at 7.8 hour and 8 day, respectively, resulting in
95 mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS)
96 concentrations of 1300-1500 mg/L and 1100-1200 mg/L. The initial MLSS concentration used in
97 this experiment differed depending on purposes and days when concentrated activated sludge was
98 taken, i.e., 4,000 mg/L in the experiment 2.3 and 6,000-11,000 mg/L in the experiment 2.4. Before

99 the HPJD experiments, the sludge was subjected to intense mixing to completely detach any
100 bubbles generated mainly by denitrification.

101 **2.3. Effect of the HPJD's operating conditions on the degree of solubilization**

102 To determine the effect of manner of sludge supply on the degree of activated sludge
103 reduction, three types of HPJD configuration were used, as shown in Figure 2. The three methods
104 used to supply activated sludge were, Run 1: supply of activated sludge in the horizontal direction
105 by high-pressure jet, Run 2: supply of activated sludge in the horizontal and vertical upper
106 directions, Run 3: supply of activated sludge in the horizontal and vertical lower directions. The
107 latter direction employs the suction of activated sludge from the bottom of the HPJD (Run 2) and
108 the top of the HPJD (Run 3). In addition to the aspiration effect from Run 2, the configuration in
109 Run 3 allows air to be sucked downwards as fine bubbles, which potentially dissipates by cavitation.
110 The experiment was initiated by the supply of 80 L of activated sludge to the nozzle in Run 1 and
111 40 L of activated sludge to both the nozzle and the lower or upper ports in Run 2 and Run 3,
112 respectively. The discharged activated sludge from each HPJD was subjected to a maximum of five
113 HPJD treatments. After each treatment, 50 mL of the activated sludge was taken for measurement of
114 MLVSS whereas the rest of the samples were used for further analysis. Microorganism cell viability
115 in the treated activated sludge, as an indicator of the degree of activated sludge solubilization upon
116 HPJD treatment, was performed by LIVE/DEAD staining according to manufacturer instructions.
117 The viability was calculated by dividing the damaged cell area (PI staining) by both the intact
118 (SYTO9 staining) and the damaged cell areas using Daime imaging software [28].

119 2.4. Optimization of the injection ratio R of the activated sludge

120 For the optimization of the HPJD's operational conditions, the efficiency of sludge
121 solubilization was evaluated by measuring the MLSS before and after the HPJD operation. To this
122 end, the ratio of activated sludge distribution to either a port connected to a nozzle or a side port,
123 referred to as the injection ratio hereafter, was altered. The injection ratio, R , was defined as:

$$124 \quad R = \frac{a}{b} \quad (1)$$

125 where a is the amount of activated sludge supplied by the side port and b is the amount of activated
126 sludge from the nozzle. Reduction percentage of MLSS, M [%], was defined:

$$127 \quad M = \frac{\text{MLSS}_i - \text{MLSS}_a}{\text{MLSS}_i} \times 100 \quad (2)$$

128 where MLSS_i and MLSS_a are MLSS concentrations before and after HPJD treatment, respectively.

129 Solubilization rate of sludge, S [g/min] was defined:

$$130 \quad S = (\text{MLSS}_i - \text{MLSS}_a) \times Q \quad (3)$$

131 where Q is the amount of treated activated sludge in volume per minute. Microorganism cell
132 viability was calculated from fluorescence microscopy images [28].

133 2.5. Solubilization of organic compounds by HPJD treatment

134 After HPJD treatment, the dissolved compounds from the activated sludge in the bulk
135 liquid were quantified in terms of the dissolved organic carbon (DOC) and the total nitrogen (TN).
136 The treated activated sludge was centrifuged at 10,000 rpm for 5 min and the supernatant was
137 removed by filtration using a 0.45 μm membrane filter (Advantec, Tokyo, Japan). The filtrates were
138 further used in biodegradation tests.

139 **2.6. Biodegradability of the treated activated sludge**

140 The biodegradation behavior of the filtrate solubilized by the HPJD under different
141 operating conditions was evaluated by CO₂ trapping. Two hundred milliliters of non-filtered
142 suspension and filtered liquid from the HPJD treatment were separately transferred into 500 mL
143 screw-capped beakers. Subsequently, 200 mL of activated sludge samples from the Kitatama Ichigo
144 Water Reclamation Center (Tokyo, Japan) were inoculated in the beakers, followed by incubation of
145 the resultant 400 mL activated sludge samples for 6 days by supplying pure air (G1 grade). The
146 exhaust gas was evacuated and transferred to a column filled with granulated CaCl₂ to remove the
147 moisture in the exhaust gas. The exhaust gas from the column was directed to a screw-capped
148 beaker containing 250 mL of 0.5 mol NaOH, to trap the carbon dioxide (CO₂). The CO₂ generated
149 by activated sludge decomposition because of the inoculum and the organic carbon solubilized from
150 the activated sludge by HPJD treatment was evaluated by analyzing the total organic carbon (TOC)
151 concentration.

152 **2.7. Analytical methods**

153 **MLSS, MLVSS and SS** were measured according to the Standard Methods [29]. Samples
154 for water quality analysis were centrifuged at 10,000 rpm for 5 min and filtered using a 0.45 µm
155 membrane filter (A045A025A, Advantec, Tokyo, Japan). The anions (nitrite, nitrate, and phosphate
156 ions) and cation (ammonium) were analyzed using an ion chromatography system ICS1000 and
157 ICS90 (Dionex, Boston, MA). Inorganic carbon (IC), TOC and TN were analyzed by TOC-V
158 (5000A, Shimadzu, Kyoto Japan). Activated sludge was stained by LIVE/DEAD Cell Viability

159 Assays (Invitrogen, Carlsbad CA) according to the manufacturer's protocol, and examined using a
160 fluorescence microscope (BZ-9000, Keyence, Osaka Japan). The image analysis software, Daime,
161 was used to calculate the viability of microorganism cells [28]. Comparison of data was performed
162 using the t-test and Dunnett's T3 test in SPSS Statistics 20 (IBM, NY)

163

164

165 **3. Results and Discussion**

166 **3.1. Effect of manner of activated sludge supply on the degree of solubilization**

167 Changes in MLSS concentrations and the viability of microorganism cells from the
168 different activated sludge supply methods are shown in Figure 3. The activated sludge was treated
169 three times using the HPJD in three runs (Fig. 2). The application of the HPJD reduced the MLSS
170 concentrations with reduction efficiencies of 5.7%, 12.4%, and 12.2% in Runs 1, 2 and 3,
171 respectively. All the runs showed a remarkable difference in MLSS reduction before and after HPJD
172 treatment with a two-sided 95% confidence interval. Additionally, there were statistically significant
173 differences in MLSS reduction between Run 1 and Run 2, and Run 1 and Run 3 whereas there was
174 a marginal difference in MLSS reduction between Run 2 and Run 3. The lowest viability of
175 microorganisms was obtained in Run 3 (Fig.3 (b)). The viability of activated sludge in Run 3
176 decreased to 42.3% whereas that before the HPJD application was as high as 81.3%. On the other
177 hand, the viability before and after HPJD treatment upon the application of only horizontal jet flow
178 via a nozzle (Run 1) did not show a significant difference. This indicates that the combination of

lateral jet flow of activated sludge with vertical flow from above in the HPJD enhanced the decrease in MLSS concentration more than lateral jet flow on its own. Given these results, the horizontal jet flow of activated sludge with vertical flow from above in Run 3 resulted in the most effective activated sludge solubilization and the most damage to microorganism cells. The vertical flow of activated sludge from above to the HPJD, where a jet flow of activated sludge was introduced, is likely to generate fine bubbles and to create turbulent flow because of the aspiration of air, resulting in more damage to the microorganism cells. The formation of fine bubbles and a turbulent environment was additionally confirmed in the HPJD made of acrylic pipes, where only water was supplied from both ports (Data not shown). Fine bubbles were formed and diffused to whole part of the inner pipe only when water was conveyed from above and a nozzle (Run 3) while they did not occupy the whole part of the pipe in Run 1 (Data not shown). This observation consolidates the trend that the HPJD geometry with two ports from above and a nozzle intensifies mixing of activated sludge with fine bubbles, resulting in higher MLSS reduction performance and more bacterial cell destruction as shown in Figure 3. Some studies have reported that microbubbles increase the decomposition of microorganism cells, which supports our observation [30]. A discrepancy between the reduction in cell viability and the MLSS concentration (c.a. 39% vs. 12% in Run 3) likely stems from the propensity of the HPJD to damage microorganism cells: the degree of cell damage by the HPJD was not as extensive as that in other treatments, e.g. ozone treatment. The HPJD damages microorganism cell walls and membranes but does not decompose or oxidize the cell structure completely.

199 3.2. Optimization of the injection ratio R

200 From Figure 3, both the supply of activated sludge from the nozzle and the side port with
201 air (Run 3) was most effective for the solubilization of microorganisms. Activated sludge reduction
202 efficiency likely depends on the injection ratio R and a change in R is expected to increase the
203 treatment efficiency per input energy. The effect of injection ratio R with different iterations of
204 HPJD treatment on the percentage of MLSS concentration reduction is shown in Figure 4. The
205 reduction in MLSS percentage increased with an increase in the number of HPJD treatments for all
206 the applied injection ratios. At a R of 1, a reduction of 15.1% after the first HPJD application was
207 obtained and this increased to 22.9% after the third HPJD application. A further decrease of 27.1%
208 was obtained after the fifth HPJD application. The first treatment of activated sludge was most
209 effective for the reduction of activated sludge and the effect of HPJD on MLSS reduction correlated
210 with the number of treatments. This result suggests that a single HPJD application rendered
211 sufficient activated sludge reduction when installed in a wastewater treatment plant. At a R of 3, the
212 highest MLSS reduction of 40% was obtained. However, the MLSS reduction percentage decreased
213 at a R of 5 with higher standard deviations regardless of the number of HPJD treatment. At a R of 5,
214 the aspiration effect was limited because the side port of the HPJD was fragmentarily filled with an
215 activated sludge suspension. The lowest reduction percentage of MLSS was obtained at a R of 10.
216 In this case, the side port of the HPJD was completely soaked with an activated sludge suspension
217 so that the aspiration effect observed for the injection ratios of 0, 1, and 3 was marginal. The
218 soaking effect led to broad standard deviations in terms of reduction of MLSS (Figure 4) and the

amount of reduced activated sludge **per unit time** (Figure 5). The HPJD plausibly generated strong shear stress in the activated sludge that was supplied by the horizontal jet flow and the vertical flow containing the air. However, this effect was compromised at higher injection ratios such as a R of 10. Mixing the activated sludge with air was done to generate and dissipate fine bubbles, but overloading the activated sludge from the side port limited the inclusion of air with activated sludge, leading to a decrease in MLSS reduction efficiency. This result assists in determining the required conditions for air aspiration from the side port when a HPJD is installed in a WWTP. Throughput of activated sludge with a HPJD was also investigated. **Solubilization rate of activated sludge as a function of R** is shown in Figure 5. The highest throughput was obtained at R of 3. Meanwhile, an injection ratio below or above 3 rendered lower throughput of activated sludge solubilization, the trend of which agrees with the MLSS reduction efficiency as shown in Figure 4. Therefore, the optimum conditions, i.e., at an injection ratio R of 3 with horizontal jet flow via a nozzle and vertical activated sludge dosing from the elevated side port, provided a maximum 40% reduction of MLSS in the activated sludge with the highest rate of activated sludge solubilization.

3.3. Effect of HPJD treatment iterations on the viability of microorganisms

Activated sludge samples were obtained after every HPJD application R and subjected to LIVE/DEAD[®] staining. The viability of microorganisms after the HPJD operation at different injection ratios R is shown in Figure 6. HPJD treatment at an injection ratio R of 3 gave the highest percentage of damaged microorganisms. The proportion of damaged microorganisms did not significantly increase after three HPJD treatments, and this was consistent with the trend shown in

239 Figure 6. Identification of intact and damaged bacteria in activated sludge is yet to be performed.
240 Nevertheless, the effectiveness of cell damage on an anti-bacterial surface has been reported to
241 depend on the cell wall and membrane structure, especially the thickness of the peptidoglycan, such
242 as in Gram-positive and Gram-negative bacteria [31]. Investigation on the fate of microbial
243 community by HPJD treatment will be carried out in future.

244 **3.4. Dissolved components of treated activated sludge**

245 The increases in eluted DOC and dissolved TN (DTN) concentrations after HPJD
246 application as a function of an injection ratio R are shown in Figure 7. The DOC and DTN
247 concentrations increased with an increase in HPJD treatment number at injection ratios R of 1, 3, 5
248 and 10; however not at 0. The incremental trend except R of 0 was consistent with the result of
249 MLSS reduction percentage in Figure 4. The eluted DOC concentration at R of 3 was the highest
250 among the applied injection ratios. The ratio higher than 5 decreased eluted DOC and DTN
251 concentrations after the HPJD treatment (Figure 7 (a)), the trends of which are also consistent with
252 those on MLSS reduction efficiency (Figure 4) and viability of microorganisms (Figure 6). The
253 congruency indicates dissolution of cell envelopes of microorganisms by HPJD treatment and the
254 corresponding release of intracellular compounds into the bulk liquid. A series of the trends were
255 most pronounced at R of 3. On the other hand, when volume of activated sludge supplied from the
256 side port was excessive, i.e., at R higher than 5, the effect of MLSS reduction performance became
257 limited, resulting in lower amounts of DOC and DTN dissolutions into the bulk liquid as shown in
258 Figure 7.

259 3.5. Biodegradability of the treated and activated sludge

260 The biodegradability of the dissolved cell constituents from the damaged microorganisms
261 was investigated. The integrated value of CO₂, obtained from the sum of HPJD-treated activated
262 sludge and endogenous decay, is shown in Figure 8. The integrated CO₂ values of the activated
263 sludge treated by the HPJD were much higher than that derived from the DOC, suggesting that
264 microorganism debris was eluted and eventually used as a substrate by the intact microorganisms in
265 the activated sludge. In this biodegradability test, the injection ratios R of 0, 3, and 5 were
266 comparatively high. The DOC concentration was negligible in all the biodegradability experiments,
267 indicating that slow-degrading or non-degradable organic carbon was not dissolved or did not
268 accumulate in the beakers. Given these results, activated sludge reduction by the HPJD probably did
269 not affect the organic carbon removal efficiency although the treated sludge and the liquor were
270 recycled to the activated sludge. Foladori et al (2010) reported that a high pressure homogenization
271 (HPH) technology, analogous to HPJD regarding the principle using cavitation for activated sludge
272 reduction, disrupts intact bacterial cells with intensity not as high as ozonation [19]. However,
273 ozonation intensively decomposes bacterial cell wall and membrane, which potentially produces
274 inert materials and organic carbon to be wasted and removed [32]. Despite less intensive disruption
275 of intact bacterial cells than ozonation, HPJD decomposes partially bacterial cells, releasing organic
276 compounds which can be biologically mineralized. Hence, this device is expected to avoid release
277 of inert materials in an activated sludge system more than other activated sludge reduction
278 technologies. A pilot-scale activated sludge system incorporating an HPJD is under operation,

279 which is to be reported.

280 **3.6. Estimation of cost reduction by the use of a HPJD**

281 The estimated operational cost of a HPJD was compared with other activated sludge
282 reduction technologies. Ozonation is the most widely used method, and gives a 10–60% reduction
283 efficiency as a TSS unit in a semi-batch experiment [32]. The performance of activated sludge
284 reduction by ozonation reportedly increases depending on the volume of dosed ozone, and the
285 reduction ranges from 30% at an ozone dose of 0.03–0.04 g-O₃/g-MLSS [33] to 58.9% at an ozone
286 dose of 1.2 g-O₃/g-MLSS [34]. Therefore, the input energy for the treatment of activated sludge was
287 compared as an operational cost. Ozonation requires 72.0 kJ/g-O₃ (20 Wh/g-O₃) [35, 36]: for
288 example, in the study by Saltaywin *et al.* they used 2.16 kJ/g-MLSS energy for a 30% activated
289 sludge reduction [33]. On the other hand, the HPJD developed in this study relies on a high pressure
290 pump, which requires 3.6 MJ/hr for the treatment of activated sludge. Assuming the optimum
291 condition obtained in this study, i.e. an injection ratio R of 3 (1.5 and 4.5 L/min by lateral jet flow
292 with a nozzle and from the side port, respectively) for the initial MLSS concentration of 8,500 mg/L
293 as a median MLSS value (6,000–11,000 mg/L in our experiment) and MLSS reduction efficiencies
294 of 40% by HPJD treatment three times, the operational cost is estimated to be 3.53 kJ/g-MLSS.
295 This cost is thus lower than that of other activated sludge reduction technologies and is comparable
296 to the ozone technology given in reference [4], as shown in Table 1. Given this estimation, a single
297 application of the HPJD to reduce the activated sludge, and that achieves a 30% MLSS reduction
298 (Figure 4), will have an estimated operational cost of 1.18 kJ/g-MLSS, which could thus be the

most cost-effective activated sludge reduction technology. Due to limited consistent data available for comparison in terms of MLSS reduction performance, throughput X [g-MLSS/min] was defined with this equation:

$$X = \frac{S}{N} \quad (4)$$

where S [g/min] is the solubilization rate of sludge defined as Eq. 3 and N is the number of treatment. Ozonation technologies cannot be estimated because of continuous treatment for solubilization. Instead, a HPH [37, 38] was subject to the comparison. It turned out that a HPJD has a higher throughput of activated sludge, 3.2-7.2 times as high as a HPH with the identical sludge reduction performance. This is because of the side port installed in the HPJD where sludge can be supplied from the two individual ports with aspiration and via a nozzle. Given that a HPH was successfully implemented in a pilot scale membrane bioreactor for sludge reduction [27], a HPJD could be an alternate to reduce sludge production. Likewise, the HPJD can be applicable to the installation to the return activated sludge line of a WWTP. Furthermore, the HPJD still has room for improvement in terms of cost-effectiveness. Given that a reiteration number of HPJD treatment, especially more than three times, did not substantially improve activated sludge reduction (Figure 4), excessive treatment causes extra energy input and cost. More notably, a scale-up of the high-pressure pump will have cost advantages. Hence, cost benefits will result from the practical scaling up of a HPJD (Table S1).

319 4. Conclusion

320 A HPJD was applied to excess activated sludge to investigate its performance, the
321 conditions of microorganism treatment and the biodegradability of the eluted cell constituents. The
322 degree of activated sludge reduction increased with an increase in the number of HPJD treatments.
323 It was demonstrated that an injection ratio R of 3 is the optimum value for maximum performance.
324 The HPJD treatment damaged microorganisms with the degree dependent on R and the number of
325 treatments and, accordingly, enhanced release of the cell components and intracellular compounds.
326 These released compounds were biologically degraded by activated sludge, implying that sludge
327 reduction by HPJD does not exacerbate the organic carbon removal efficiency when the treated
328 sludge is returned to the main stream of a WWTP. The estimation on cost and performance for
329 HPJD operation underscores that HPJD is more cost-effective and higher throughput for sludge
330 treatment than other conventional technologies, which is promising for its application in a
331 recirculation line from a secondary clarifier to a main activated sludge basin. The incorporation of
332 the HPJD into a pilot-scale conventional activated sludge system is currently underway.

333

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Table 1. Summary of the reduction cost for each sludge solubilization technology

Sludge solubilization technology	Preparation mode	Ozone dosage [g-O ₃ /g-MLSS]	Solubilization efficiency [%]	Flow rate [L/min]	Throughput [g-MLSS/min]	Required energy [kJ/g-MLSS]	References
Ozone	Semi-batch	0.03–0.04	30	-	-	2.16 – 2.88	[33]
Ozone	Semi-batch	0.05	30	-	-	3.6	[39]
Ozone	Semi-batch	0.1	45	-	-	7.2	[40]
Ozone	Semi-batch	0.57–1.09	50–56	-	-	41.1 – 78.5	[41]
High-pressure Homogenization	Semi-batch	-	41.17	0.5	2.37	4.22	[37, 38]
HPJD	Semi-batch	-	30 - 40	6.0	6.97 – 15.3	1.18 - 3.53*	This study

* These values were obtained assuming that HPJDs of the same dimensions as those employed in this study are operated once or three times at an injection ratio R of 3.

Figure 1. Conceptual high-pressure jet device, the HPJD. Some activated sludge is transferred to a nozzle by a high-pressure pump and conveyed horizontally to the HPJD whereas the other is drawn from the top (side) port and mixed with air.

Figure 2. Activated sludge injection patterns in the HPJDs: in Run1 the activated sludge was only injected into a HPJD horizontally by a high-pressure pump; in Run 2 the activated sludge was drawn from below in conjunction with being conveyed by a lateral jet flow via a nozzle in the HPJD; and in Run 3 the activated sludge was dropped vertically from the side port and contacted the sludge conveyed by jet flow via the nozzle.

Figure 3. Effect of injection patterns on the HPJD: changes in (a) the MLSS concentrations; (b) the viability of the microorganism cells; and microscopic images of LIVE/DEAD[®] staining (c) before HPJD treatment; and (d) after three HPJD treatments (Run 3). The green and red areas represent intact and damaged microorganisms, respectively. Values of MLSS and viability denoted with *, **, and *** are significant at $P < 0.05$ by Dunnett's T3 test.

Figure 4. Effect of the injection ratio R with different iterations of HPJD treatment on the reduction of the MLSS concentration.

Figure 5. Effect of the injection ratio R with different iterations of HPJD treatment on the solubilization rate of sludge

Figure 6. Viability of microorganisms with different HPJD treatment iterations. There were no significant differences in the values before HPJD treatment ($P > 0.05$, Dunnett's T3 test). Viability percentages denoted with * and ** are significant at $P < 0.05$ by Dunnett's test.

Figure 7. Effect of injection ratio R with different iterations of HPJD treatment on: (a) the increase in DOC concentration and (b) the increase in dissolved total nitrogen concentration of the supernatant from the pre-treatment activated sludge.

Figure 8. Effect of treated activated sludge at different injection ratios R on the amount of decomposition by the activated sludge.