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A lab scale activated sludge sequencing batch reactor (SBR) was used to treat tank truck cleaning (TTC) wastewater with different operational strategies (identified as different stages). The first stage was an adaptation period for the seed sludge that originated from a continuous fed industrial plant treating TTC wastewater. The first stage was followed by a dynamic reactor operation based on the oxygen uptake rate (OUR). Thirdly, dynamic SBR control based on OUR treated a daily changing influent. Lastly, the reactor was operated with a gradually shortened fixed cycle. During operation, sludge settling evolved from nearly no settling to good settling sludge in 16 days. The sludge volume index (SVI) improved from 200 to 70 mL.gMLSS^{-1} in 16 days and remained stable during the whole reactor operation. The average soluble chemical oxygen demand (sCOD) removal varied from 87.0 to 91.3% in the different stages while significant differences in the food to mass ratio were observed, varying from 0.11 (stage I) to 0.37 kgCOD.(kgMLVSS.day)$^{-1}$ (stage III). Effluent toxicity measurements were performed with *Aliivibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella subcapitata*. Low sensitivity of *Aliivibrio* was observed. A few samples were acutely toxic for *Daphnia*. 50% of the tested effluent samples showed an inhibition of 100% for *Pseudokirchneriella*.

Keywords: industrial wastewater; *Daphnia magna; Pseudokirchneriella subcapitata*; *Aliivibrio fischeri*; sludge volume index

Introduction

Industrial wastewater treatment became an important activity on industrial sites because of stringent environmental standards. These standards force companies to invest in wastewater treatment to minimize negative environmental impact by the produced waste. Biological wastewater treatment with activated sludge is an important treatment step to reach dischargeable effluent standards. Mostly it forms one-step in a cascade of different treatment units. For the design and operation of the biological treatment, different criteria are important: operational cost, footprint, flexibility to variable influent

composition and volume, easy process control and settling characteristics of the activated sludge [1]. These parameters are influenced by the configuration of the wastewater treatment plant. The two most common configurations are the conventional continuous flow activated sludge (CAS) reactor and the sequencing batch reactor (SBR). Although the first activated sludge reactor was operated by a fill and draw principle, the continuous flow reactor is most common in industry [2]. However, there are significant benefits attributed to the SBR in comparison to the CAS system. (i) Dynamic control of the reactor is possible [3,4]. (ii) Higher removal efficiencies can be achieved [5–7]. (iii) Faster adaptation to difficult degradable components [8–10]. (iv) Lower operational cost [11].

The feeding pattern in a CAS system is widely known to be sensitive for the development of poor settling characteristics and filamentous bulking sludge [12–14]. Houtmeyers et al. [15] operated lab scale reactors under regimes of continuous and intermittent feeding of substrate (glucose as main carbon source). Continuously fed systems rapidly resulted in the development of filamentous bacteria and bulking of the sludge. Intermittently fed systems on the other hand did form good settling sludge. The same results were obtained by Verachtert et al. [16] by using nutrient broth, acetate and starch. Van den Eynde et al. [17] confirmed this with brewery, petrochemical and dairy wastewater. The same findings were also observed by Caluwé et al. [18] during the treatment of tank truck cleaning (TTC) wastewater. Ciğgin et al. [19], Cubas et al. [20] and Martins et al. [21] investigated the influence of the feeding pattern during the treatment of starch, bactopeptone and acetate respectivly. The shorter the feeding period, the better the settling characteristics. As a general conclusion it was stated that a feast/famine regime in an SBR supresses the development of filaments and stimulates the growth of floc forming bacteria.

Different full scale industrial applications of the SBR technology are described in literature [1]. The SBR has been proven to be successful for the treatment of winery wastewater [11], brewery [22], landfill leaches [23], tannery [24], paper mill [25], slaughterhouse [26] and road/rail car cleaning [27]. In these references, a fixed SBR cycle is used. Due to this, optimal use of the SBR is not possible. To respond to variable influent flows, dynamic SBR reactor control can result in a more efficient use of this type of reactor. Different sensor signals i.e. dissolved oxygen, pH and oxidation reduction potential can be used [3,28]. TTC wastewater is highly variable because it can contain a wide range of cargo like food, petrochemicals and hazardous chemicals [29,30]. Due to this, dynamic SBR operation would be the perfect reactor for an efficient treatment.

Currently, water quality standards of discharged wastewater are not only focussing on chemical parameters but also on biological or ecological quality. Since the implementation of the European Water Framework Directive (2000/60/EC) ecotoxicological analysis gained more attention. Whole effluent toxicity (WET) testing involves different toxicological tests and is used to gain a broad view of the effluent quality. Different studies clearly stressed the importance of ecotoxicological effluent assessment since they showed that a good chemical quality not always corresponds with a good ecotoxicological quality. For example De Schepper et al. [31] and Dries et al. [32,33] showed the importance of ecotoxicological analyses for the effluent of TTC wastewater. Although a good chemical effluent quality was obtained within the discharge standard, high toxicity against bacteria, daphnias' and algae was observed.

In this research, a lab-scale SBR was investigated as an alternative for the commonly used CAS system to treat TTC wastewater. The main research objective is to determine if pulse fed activated sludge reactors form a positive contribution for the

treatment of TTC wastewater. During the experimental period, a dynamic SBR control was evaluated for the treatment of TTC wastewater. A dynamic process control based on the oxygen uptake rate (OUR) was used to treat a highly variable wastewater. Daily new wastewater was fed to the reactor to challenge the activated sludge and the dynamic process control. Furthermore, ecotoxicological effluent assessment was executed with *Aliivibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella subcapitata* to compare those results with the chemical effluent quality.

The following research questions will be answered: how will activated sludge settling characteristics and morphological structure evolve if the feeding pattern of the seed sludge is changed and when highly variable wastewater is fed to the reactor? Can activated sludge handle highly variable influent compositions and concentrations typical for TTC wastewater? Is a good chemical and ecotoxicological effluent quality reached? Is a dynamic process control, based on the oxygen uptake rate possible for this type of wastewater?

Materials and methods

Reactor setup and operation

In this research a lab-scale SBR was used to treat industrial wastewater from a TTC company. The reactor had a height of 400mm, an internal diameter of 230mm (H/D = 1.74), a working volume of 13L and a volume exchange ratio (VER) of 23%. Before feeding of the reactor, influent was pumped into an influent storage tank with a GARDENA 6000 pump (Gardena, The Netherlands). This tank was able to store exactly 3L of wastewater. After filling the tank, an electric ER-PLUS® (Watts Water Technologies, The Netherlands) valve was used to transfer the 3L of wastewater from the storage tank to the reactor in a very short time (2 minutes). Sludge was kept into

suspension with a Heidolph® RZR 2020 mechanic mixer (Heidolph Instruments GmbH & Co. KG, Germany) and the sludge was aerated with a Super Fish Koi Flow 60 (Aquadistria, China) air pomp which was connected to a ceramic air disc diffuser (Angel Aqua, Korea) with a diameter of 130mm.

During operation of the reactor, dissolved oxygen (DO) (Hach LDO sensor, Hach, United States), oxidation-reduction potential (ORP) and pH (both from Jumo TecLine) were continuously monitored. During the aerobic steps, the DO level was controlled between 1.5 and 5 $mgO_2.L^{-1}$. A sludge retention time (SRT) of 30 days was applied during the whole experimental period which was the same as in the full scale industrial installation. The reactor was operated at room temperature with an average temperature of 24.3°C with a standard deviation of 1.8°C. To satisfy the nutrient requirement, nitrogen and phosphorus were added to the wastewater as KNO_3 and K_2HPO_4 to ensure the ratio of COD:N:P to be 100:5:0.8 [30]. Nitrate was used as nitrogen source instead of the commonly used NH_4Cl to avoid nitrification.

The reactor was controlled with a programmable logic controller (PLC) (Siemens, Germany), type CPU 319-3PN/DP. Configuration of this PLC was done with Siemens Simatic Step7 software. LabView™ software from National Instruments (Austin – Texas, United States) was used as an interface to control the reactor, visualization of the process and data transfer to MS Excel.

Seed sludge from the TTC company was used to start up the lab scale reactor. The industrial treatment plant is a CAS system, so the sludge was adapted to continuous feeding. The lab scale operation, with a total duration of 162 days, consisted of 4 strategies.

Stage I: adaptation period (30 days)

After inoculation of the reactor with the seed sludge an adaptation period of 1 SRT (30 days) was given to the sludge to adapt to the new feeding pattern. During this period, a fixed reactor cycle was used with a total length of 1 day. The reactor operation times are shown in Table 1. In stage I, two different influent batches were used to feed the activated sludge. The first batch was used from day 1 to 23, the second batch from day 24 until 30. The composition of the two influent batches is shown in

Table *2*.

Stage II: dynamic reactor operation (10 days)

After adaptation of the sludge to the new feeding pattern (stage I), a more dynamic reactor operation was imposed based on the oxygen uptake rate (OUR) of the sludge. During aeration, data pairs of time and oxygen were collected in LabView. During the air off period, when the oxygen concentration in the reactor was between 1.5 and 5 $mgO_2.L^{-1}$, the data pairs were stored in an array. When the DO concentrations reached the lower level of 1.5 $mgO_2.L^{-1}$, aeration was started again and a slope calculation was done for the collected data pairs. The calculated slope corresponds to the OUR and was compared with an OUR value that was set as the endogenous OUR by the researchers (the OUR threshold was examined and adjusted if necessary). When the actual OUR of the activated sludge was lower than the imposed value, reactor operation automatically switched to the next step (i.e. sludge withdrawal followed by settling and effluent discharge).

During stage II, influent batch 2 was fed to the reactors, as shown in

Table *2*. The reactor was operated for 10 operational days in this stage with the same influent.

Stage III: variable influent composition (62 days)

During stage III, a new influent sample was taken every day at the TTC company and fed to the reactor. These variable influents (55 in total) were treated using a dynamic OUR control strategy. The reactor was running for 62 days in this regime to test the flexibility of the activated sludge to a changing organic loading rate (OLR), food to microorganism ratio (F/M) and a changing influent composition. The average influent chemical oxygen demand (COD) was 1756 $mgO_2.L^{-1}$ with a standard deviation of 801 $mgO_2.L^{-1}$. The endogenous OUR set point was changed during operation to obtain a more or less constant specific OUR (sOUR) between 2.5 and 3.8 $mgO_2.(gVSS.h)^{-1}$.

Stage IV: fixed reactor operation (59 days)

During the last stage, a fixed operation time was used. Influent composition and concentration were kept constant but the time of the aerobic reaction was gradually shortened. As a result, the total cycle time also decreased. During this stage, settling time was also lowered to 35 minutes as shown in Table 1. Three different influent batches were fed to the reactor during stage IV (

Table *2*).

Ecotoxicological analyses

To evaluate the ecotoxicological quality of the influent and effluent, the following acute toxicity tests were performed: (i) the 72 h algal growth inhibition test [34] using *Pseudokirchneriella subcapitata* (ii) the 48 h *Daphnia* immobilization test [35] using *Daphnia magna* (iii) the 30 min bacterial bioluminescence inhibition test [36] using *Aliivibrio fischeri.*

The algae cultivation was performed as described in the OECD 201 guideline. The growth medium for *Pseudokirchneriella subcapitata* used in the experiments had the following composition: 0.77 mM $NaHCO_3$, 0.28 mM NH_4Cl, 2 mM $CaCl_2.2H_2O$, 0.5 mM $MgSO_4.7H_2O$, 0.01 mM K_2HPO_4, and 1 mM EPPS (N-2-hydroxyethyl-piperazine-N-3-propane sulfonic acid) buffer. The final pH of the medium was 8.0 ± 0.2. The medium was autoclaved in 1-L flasks for 20 min at 121°C and pre-equilibrated with 1 ml of sterile trace metal solution (0.3 mM $FeCl_2.6H_2O$, 3 mM H_3BO_3, 2.1 mM $MnCl_2.4H_2O$, 0.0063 mM $CoCl_2.6H_2O$, 0.029 mM $Na_2MoO_4.2H_2O$, and 0.27 mM $Na_2EDTA{\cdot}2H_2O$ for at least 24 h before inoculation with algae. All chemicals were purchased from VWR International with a reagent grade and ultrapure water (resistivity, 18.2 M; Milli-QTM; Millipore) was used for preparation of the media. The culture growth at 22°C and was illuminated with 4440-8880 lux (400-700nm) under a 14/10-h light/dark photoperiod in a Snijders scientific Micro Clima Incubator (Snijders Labs, The Netherlands). Cultures were stirred at 100 rpm to provide oxygen with a GFL 3012 shaker (GFL Gesellschaft für Labortechnik mbH, Germany). Algae inhibition measurement happened in 48 well plates with algae that were in exponential growth phase. The outer row of the plate was filled with ultrapure water to avoid natural evaporation and dry up of the wells. Each sample was measured in triplicate and blanks

in sextuple. In total six samples and one blanc (in sextuple) were measured in each plate. Effluent samples of the reactors were measured in a ratio of 90% sample and 10% of dilution water, influent samples were 50% diluted. Only a single concentration for each sample was tested which is also the guideline in the Flemish environmental permit for discharge waters.

The *Daphnia magna* cultivation was performed in the standard OECD TG202 cultivation medium for conducting the acute immobilization test [35]. The cultivation medium consisted of the following chemicals: 2 mM $CaCl_2.2H_2O$, 0.5 mM $MgSO_4.7H_2O$, 0.77 mM $NaHCO_3$, and 0.077 mM KCl. Three times a week, the cultivation medium was renewed and *Daphnia* were fed with 400.10^6 algal cells/L. The test condition was maintained at 21.1°C under a 14/10h light/dark cycle. For all experiments, maximum 24h-old *Daphnia* were used that were isolated after birth. Always 8mL of solution was preserved for each *Daphnia*. In total, 5 *Daphnia* were incubated with each sample and every sample was measured in triplicate. Effluent samples were diluted 10% with cultivation medium, influent samples 50%. Similar to the algae growth inhibition test, only a single concentration for each sample was tested (similar to the guideline in the Flemish environmental permit).

Aliivibrio fischeri inhibition measurements were done using a commercial test with freeze-dried *Aliivibrio* (BioTox™ Kit from Aboatox Oy, Finland). The test protocol involved combining 500 µL of sample (influent/effluent) adjusted with salinity of 2% NaCl, with 500 µL of activated bacteria what makes a dilution of 50% (only a single concentration for each sample was tested). After a contact time of 30 min at 15°C, the decrease of light intensity was measured with a Junior LB 9509 portable tube luminometer (Berthold Technologies GmbH & Co. KG, Germany). The inhibitory

effect is compared to a negative control (demineralised water with 2% sodium chloride). Out of this, the percentage inhibition is calculated.

Chemical analyses, sludge characterization

The following analyses were performed to characterize the influent, effluent and sludge characteristics: COD, DOC, pH, Cl^-, conductivity, PO_4^{3-}-P, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI) and activated sludge morphology.

COD was measured with Hanna Instruments (HI) (Belgium, Temse). COD Tests HI 93754A-25 (low range) and HI 93754B-25 (medium range) tubes were used with a detection limit of 150 $mgO_2.L^{-1}$ and 1500 $mgO_2.L^{-1}$ respectively. The chloride concentration was measured with the HI 3815 test kit and ammonia with the HI 93715-01 test kit, both from Hannah Instruments. pH and conductivity were measured with a HI 9023 microcomputer pH meter and a HI 9033 multi range conductivity meter respectively. Phosphorus was measured with PhosVer3 powder pillows from Hach Lange (Belgium, Mechelen). Dissolved Organic Carbon (DOC) was analysed during the experiments with a Sievers InnovOx Laboratory Total Organic Carbon Analyzer (General Electric Company, Colorado, United States).

If necessary for the analyses, samples were filtered before they were analysed using glass microfiber filters of 1.2µm from VWR (Belgium, Leuven).

MLSS, MLVSS and SVI were measured in accordance with the procedures described in Standard Methods for the Examination of Water and Wastewater [37]. Settling characteristics were determined with a 1L graduated cylinder with a height of 34.5 cm and an internal diameter of 6.1 cm. Activated sludge morphology was observed with a Motic BA 310 microscope by using an EF-N Plan 10x0.25 ocular.

Measurements were always done at the end of the aeration phase, so sludge was always in an endogenous state.

Results and discussion

Sludge characteristics and reactor conditions

The evolution of MLSS and MLVSS concentration in the reactor is shown in Figure *1*. During stage I a decrease in the MLVSS concentration was observed, from a VSS concentration of 4.5 $g.L^{-1}$ to 2 $g.L^{-1}$ in 16 days. During this period, F/M increased from 0.06 to approximately 0.13 $kgCOD.(kgMLVSS.day)^{-1}$. During stage II a constant ML(V)SS concentration and F/M ratio (0.24 $kgCOD.(kgMLVSS.day)^{-1}$) were observed. The OLR during stage I and II was 0.25 ± 0.01 $kgCOD.(m^3.d)^{-1}$ and 0.37 ± 0.06 $kgCOD.(m^3.d)^{-1}$ respectively.

During stage III a variable F/M was observed due to a fluctuating COD influent composition. The F/M ratio varied between 0.1 $kgCOD.(kgMLVSS.day)^{-1}$ and 0.68 $kgCOD.(kgMLVSS.day)^{-1}$. Fluctuations in the OLR between 0.13 and 1.09 $kgCOD.(m^3.d)^{-1}$ with an average of 0.51 ± 0.30 $kgCOD.(m^3.d)^{-1}$ were observed. During stage IV, an increased ML(V)SS concentration and F/M (0.45 $kgCOD.(kgMLVSS.day)^{-1}$) were observed due to the shortening of the cycle time. The OLR increased to 1.16 $kgCOD.(m^3.d)^{-1}$. The F/M values reached in stage III and IV were significantly higher than in the industrial installation (0.08 $kgCOD.(kgMLVSS.day)^{-1}$) and the previous stages (I and II).

Statistical analysis (results shown in table 3) shows that a significant difference in the F/M was used during the different stages (p: 0 – 0.001). The F/M ratio was the lowest during stage I and highest during stage III and were significantly different between stage I, II and III. The F/M ratio during stage II and IV was equal. The OLR

imposed to the reactor was highest during stage III and IV and was significantly different from the other two stages (p: 0 – 0.001).

The SVI after 10 minutes (not shown) and 30 minutes was measured to characterize sludge settling (Figure *2*). The seed sludge was characterised by poor settling rates. After 30 minutes settling, an SVI_{30} of 200 mL.gMLSS^{-1} was obtained with a MLSS concentration of 4.90 g.L^{-1}. This means that almost no settling occurred. Between day 10 and 14, a significant improvement in the settling was obtained with the SVI_{30} decreasing to 70 mL.gMLSS^{-1}. In stage III, the sludge was subjected to highly variable influent compositions and substrate concentrations. Sludge settling was not affected by these variations. During the whole stage, a stable SVI_{30} of 59.4 ± 6.0 mL.gMLSS^{-1} was obtained. During stage IV, an increase in the SVI_{30} occurred to 90 mL.gMLSS^{-1}. After switching to influent batch 5, SVI_{30} improved again to 80 mL.gMLSS^{-1}.

The morphology of the sludge (Figure *3*) was linked to the measured settling characteristics. The seed sludge from the industrial installation showed a very irregular shape and an open structure which explains the poor settling characteristics. Already after 10 days, it was possible to observe defined floc structures. Sludge flocs with a diameter up to 500 µm were present after 20 days. The size of these flocs corresponds to granular sludge [38]. During the next 80 days, flocs became darker and denser. Around day 100, round shaped, dark coloured sludge flocs were formed with a diameter between 250 µm and 500 µm. Nevertheless the SVI_{10}/SVI_{30} ratio never reached 1 (Figure *2*) as it is the case for aerobic granular sludge. Probably because there were still small irregular sludge flocs present.

During the whole reactor operation, morphological structure and settling characteristics were clearly related. Changing the feeding pattern resulted in an

improved and stable settling pattern, after ½ sludge age. Similar results were obtained by Caluwé et al. [39] for petrochemical wastewater. This is significantly faster than the 2 to 3 sludge age as commonly reported [40]. Several authors already described that, using synthetic easily degradable wastewater (acetate, glucose, peptone, …), sludge settling characteristics can be improved by applying pulse feeding [16,20,21,41]. The number of studies that describe this phenomenon for industrial wastewaters is however poorly low. Papadimitriou et al. [10] worked with phenol rich wastewater and Van den Eynde et al. [17] presented results with brewery, petrochemical and dairy wastewater. In both studies, a significant benefit was observed for pulse fed activated sludge with respect to the settling characteristics. The benefits of a changing feeding pattern from continuous to pulse feeding, i.e. were also clearly shown for the treatment of petrochemical wastewater with improved settling characteristics and higher sludge activities [39,42].

Reactor operation time and chemical effluent quality

During stage I, the reactor was operated with fixed cycle times (Table 1 and Figure *4* B). During every cycle a respirogram was constructed. A typical respirogram is shown in Figure *4* A. As indicated on this figure, the endogenous respiration rate started after approximately 14.5 hours. This means that the aeration period of the SBR cycle could be shortened. To obtain a shorter cycle time, a dynamic reactor operation, based on OUR control, was programmed in the reactor cycle. During stage II, an average total cycle time of 15.6h ± 1.8h was observed. Due to the dynamic reactor operation, total cycle length was shortened with an average of 8.4h. During stage III, more variation in the cycle time was observed due to the variable influent (Figure *1* (A)). The reactor was operated with an average cycle length of 18.1h ± 3.8h with a minimum time of 13.18h

and a maximum of 23.67h. During stage IV, operation times were shortened gradually from 23.6h to 10.3h as shown in figure 3 B.

The evolution of the effluent quality (sCOD and DOC) for the different stages is summarized in table 3. Detailed results of DOC removal are displayed in Figure 4 B. The effluent DOC was constant for each influent batch during stage I, II and IV but differences were observed between the stages. Stage II showed the lowest effluent sCOD. Stage IV showed the highest removal rate for sCOD and DOC. During the period with variable influents in stage III, more variation in effluent DOC was observed.

A statistical Anova test was performed to compare the obtained effluent quality (DOC and sCOD) between the different stages. Results are shown in table 3. It can be concluded that the effluent sCOD values in stage I, II and IV were comparable but the effluent of stage III was significantly different from the effluent of stage II (p: 0.01 - 0.05). Equal COD removal efficiencies were obtained for all stages ($p > 0.05$). The effluent DOC values of stage I and II were significantly different from the effluent of stage III and IV ($p < 0.001$). Although, it has to be mentioned that this is not caused by the operation strategy applied in this stage but it can be explained by the changing influent composition. Earlier research by Caluwé et al. [18] has shown the relationship between the influent composition and the effluent DOC for a similar TTC wastewater. Furthermore, it is important to note that sludge was always endogenous at the end of the aeration phase. Extending the aeration would not improve the effluent quality since all remaining DOC and COD was not biodegradable.

Dynamic SBR operation is possible using online sensor signals [3,43,44]. Dries [3] showed the implementation of a dynamic reactor control during nutrient removal from TTC wastewater. In the current study, the respiration rate (OUR) was used as a measure of the sludge activity. On Figure 4 A, different aeration rates can be observed.

After the feeding step, OUR values of approximately 40 $mgO_2.(L.h)^{-1}$ were measured. After a drop to 25 $mgO_2.(L.h)^{-1}$ a slow decrease was observed. Activated sludge shift to the endogenous aeration when all the substrate is degraded. This is indicated with the lowest aeration rates. The start of the endogenous aeration is indicated on the graph as the intersection point between the OUR curve and the OUR set point line. At this point, dynamic reactor control switches to the next step.

Activation of the real-time control strategy resulted in variable cycle lengths (Figure *4* (B)) from 13 to 24 h to handle the variable COD (Figure *1* (A)) during stage III. The F/M ratio was 4.25 times higher than in the full-scale plant which is operated as a continuous flow plant. Although care should be taken when comparing laboratory results with full-scale data, these observations suggest that changing the feeding pattern of the industrial installation would positively influence the operational capacity of the installation. Furthermore, implementation of a real-time control strategy would mean a beneficial reduction of costs since fixed aeration time could be reduced.

Ecotoxicological influent and effluent assessment

The results of the ecotoxicological measurements with *Pseudokirchneriella subcapitata* and *Daphnia magna* are shown in Figure *5* A and B respectively. 12 influent and 12 effluent samples were analyzed. The sample from day 23 represents the influent of batch 1 and an effluent sample before changing to influent batch 2. The samples from day 64 until 100 represent different influents from stage III and the corresponding effluent samples after treatment in the reactor. The samples at day 141 and 162 were taken at the end of batch 4 and 5 respectively.

From all tested influents, only two resulted in a growth inhibition lower than 100% for algae, namely the sample from day 66 and 67 with an inhibition of 93.2 ± 1.79% and 90 ± 5.0%. 6 of the 12 effluents showed an inhibition of 100% for algae. For

the other effluents, inhibition was variable between 23.8 ± 2.48% and 97.1 ± 7.8%. For *Daphnia* 100% immobilization was measured for all influent samples. Immobilization was lower than 20% for 8 effluent samples. Only for one sample (day 94), 100% immobilization was measured. Influent inhibition for *Aliivibrio fischeri* ranged between 78% and 100%. For all effluent samples, an inhibition lower than 5% was measured.

The DOC removal efficiency of all tested samples was above 86%. Interestingly, the sample with the lowest DOC removal (day 73) was not toxic to *Aliivibrio* and *Daphnia* and less harmful for the green algae compared to the other samples.

A battery of different acute ecotoxicological tests was performed to evaluate the effluent quality. All tested influents caused an inhibition of 100% against algae and *Daphnia*, for *Aliivibrio* 4 samples showed an inhibition lower than 100%. For the effluent, algae were seen as the most sensitive organism. The dynamic reactor control had no influence on the ecotoxicity of the effluent samples. Different conclusions can be made from the observed results. Firstly, variations in ecotoxicological effluent quality were observed during the whole experimental period, although DOC removal was constant. This allows the authors to conclude that there is no correlation between chemical (DOC) and ecotoxicological quality. This is also previously shown by De Schepper et al. [29]; Dries et al. [32,33] and Gartiser et al. [45]. Due to the complexity of the influent, it is not possible to relate algae toxicity to a specific cleaned chemical since the influent is a complex mixture of different chemicals. De Schepper et al. [29] investigated algae inhibition for effluent produced by TTC treatment plants and found that acetochlor was the main substance that caused inhibition of algae. In the current study, no acetochlor was cleaned during the sampling period so algae inhibition cannot be explained by this chemical. Secondly, the difference in sensitivity between algae, *Daphnia* and bacteria indicate the importance to use a battery of ecotoxicity tests to

predict the impact of an effluent. Thirdly, a dynamic reactor control was successfully implemented without affecting the effluent ecotoxicity.

Conclusion

During this research the benefits of a pulse fed activated sludge system were shown. Changing the feeding pattern resulted in a significant improvement of activated sludge characteristics. Very stable and good settling sludge was obtained, even with a highly variable influent concentration and composition.

To conclude the research questions: the results presented clearly show the benefits of an SBR for the treatment of TTC wastewater. High F/M ratios can be obtained without affecting the chemical effluent quality and settling characteristics. No inhibition of the sludge was observed, even during periods with a variable influent. Additionally effluent COD was well below the Flemish discharge limits for TTC companies (500 $mgCOD.L^{-1}$). Post treatment, such as activated carbon filtration or advanced oxidation, will be necessary to decrease the inhibition against algae. Furthermore it can be concluded that the dynamic aeration process control was an easy and successful strategy to optimize the reactor during the treatment of TTC wastewater.

Acknowledgements: PhD grant supported by the University of Antwerp. Support by the Belgian tank truck cleaning commission (CTC) was greatly appreciated.

Disclosure statement. The authors' declare that they do not have financial interest or benefit that has arisen from the direct applications of their research.

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List of tables:

Table 1: Operation times of the different steps in the SBR cycle during stage I, II, III and IV (times are shown in h).

	Stage I	Stage II	stage III	stage IV
Day	0 - 30	30 - 40	40 – 102	102 - 162
Pre aeration phase	0.25	0.25	0.25	0.25
Feeding phase	0.033	0.033	0.033	0.033
Aerobic reaction	21.7	Dynamic OUR strategy, (10 - 21.3)	Dynamic OUR strategy, (10 - 21.3)	Fixed and shortened in time (23.6 – 10.3)
Settling phase	2	1	1	0.58
Draw phase	0.033	0.033	0.033	0.033

Table 2: Physicochemical and ecotoxicological characteristics of the influent batches in the different stages of the operation of the reactor.

	Stage I		**Stage II**	**Stage III**	**Stage IV**		
Batch n°	1	2a	2b	Variable	3	4	5
Day	0-23	24-30	30-40	40 - 102	102-108	108-141	141-162
COD ($mgO_2.L^{-1}$)	1120	960		1756 ± 802	2268	1052	2122
SCOD ($mgO_2.L^{-1}$)	1034	941		/	2124	964	1986
DOC ($mg.L^{-1}$)	270	266		431 ± 208	678	488	845
pH	6.9	7.4		7.0 ± 0.4	6.8	6.6	6.6
Conductivity ($mS.cm^{-1}$)	1.6	1.6		1.43 ± 0.37	1.5	1.6	2.7
Cl^- ($mg.L^{-1}$)	350	220		/	270	200	320
NH_4-N ($mg.L^{-1}$)	10.0	8.1		/	10.0	8.2	25.6

Table 3: Physicochemical characteristics (average values) of the effluent during the different stages of the operation of the reactor. The correlation between the different stages for each parameter are indicated with the letters "a, b, ab, c and bc". Legend of the significance level: 0 – 0.001: ***, 0.001 – 0.01: **, 0.01 – 0.05: *, > 0.05: -

	Stage I	**Stage II**	**Stage III**	**Stage IV**	**Significance level**
Effluent sCOD	145.0 ± 13.5 (ab)	95.5 ± 27.5 (a)	155.8 ± 35.7 (b)	126.3 ± 19.8 (ab)	*
Removal sCOD (%)	87.0 ± 1.2 (a)	90.4 ± 2.9 (a)	88.8 ± 6.5 (a)	91.3 ± 2.4 (a)	-
Effluent DOC (mg $C.L^{-1}$)	24.5 ± 1.4 (a)	24.7 ± 2.9 (a)	40.1 ± 12.3 (b)	40.3 ± 6.3 (b)	***
DOC removal (%)	90.9 ± 0.5 (ab)	90.6 ± 1.1 "ab"	89.3 ± 6.8 (a)	92.9 ± 2.4 (b)	*
F/M ($kgCOD.kgVSS^{-1}.d^{-1}$)	0.11 ± 0.03 (a)	0.24 ± 0.02 (b)	0.37 ± 0.15 (c)	0.30 ± 0.11 (bc)	***
OLR ($kgCOD.m^{-3}.d^{-1}$)	0.25 ± 0.01 (a)	0.33 ± 0.07 (a)	0.58 ± 0.29 (b)	0.71 ± 0.36 (b)	***
Cycle duration (h)	24 ± 0 (c)	15.7 ± 1.9 (ab)	18.1 ± 3.6 (b)	13.9 ± 4.7 (a)	***

List of figures:

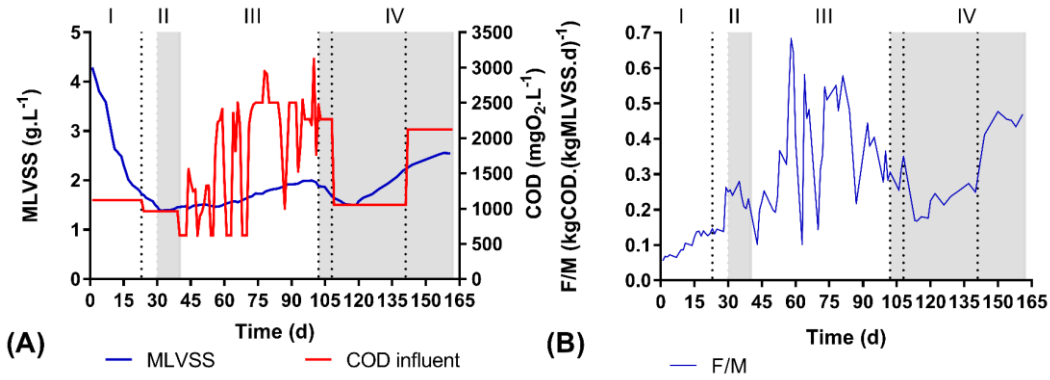

Figure 1: (A) MLVSS concentration and influent COD. (B) F/M ratio in the reactor during the whole operation period. (Vertical dotted line represents a switch to a new influent batch) (note: in stage III, a new influent batch was used every day)

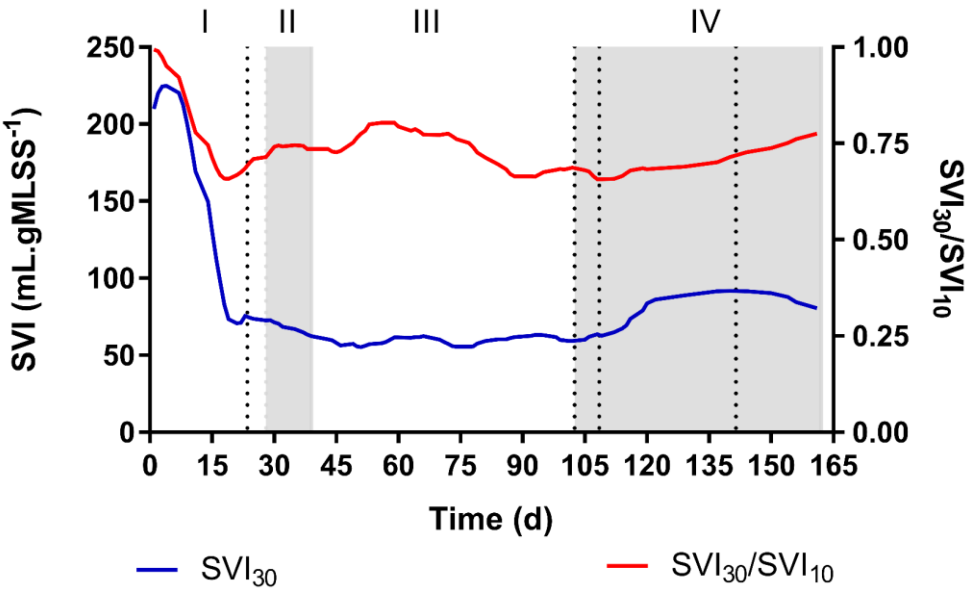

Figure 2: Evolution of the SVI_{30} and SVI_{30}/SVI_5 during the whole operation period. (Vertical dotted line represents a switch to a new influent batch) (note: in stage III, a new influent batch was used every day)

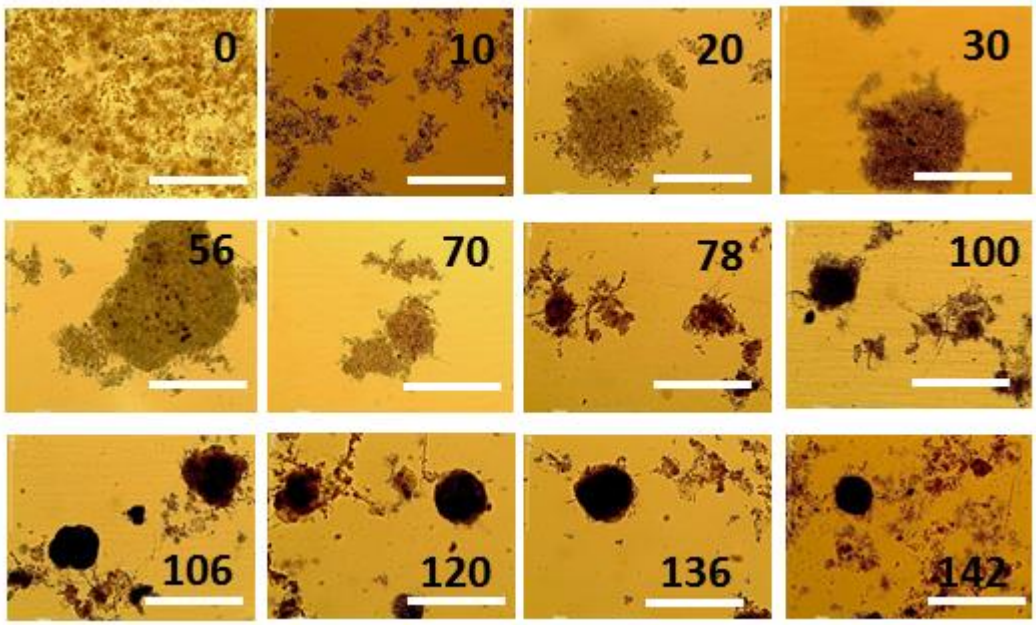

Figure 3: Morphological evolution of the sludge in the reactor. A number in each picture indicates the day of sampling. White scale bar represents 500µm.

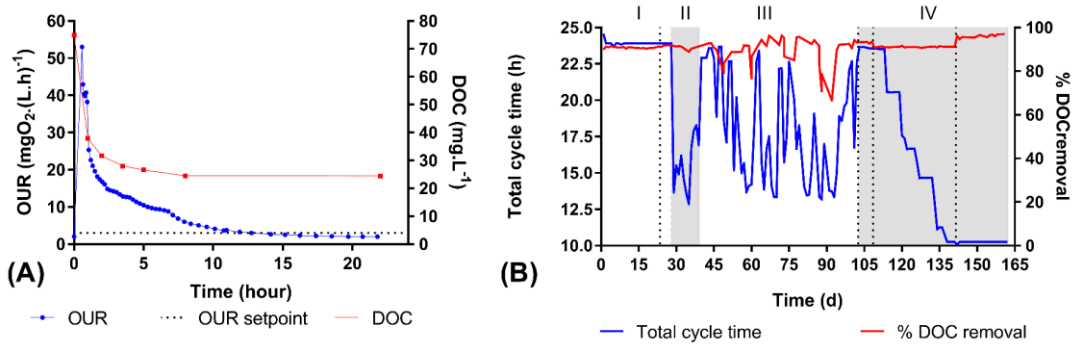

Figure 4: (A) Respirogram of the reactor at day 29, before starting stage II together with the DOC removal and the OUR set point for the endogenous aeration rate (B) evolution of the total cycle time and the DOC removal during the whole reactor operation. (Vertical dotted line represents a switch to a new influent batch) (note: in stage III, a new influent batch was used every day).

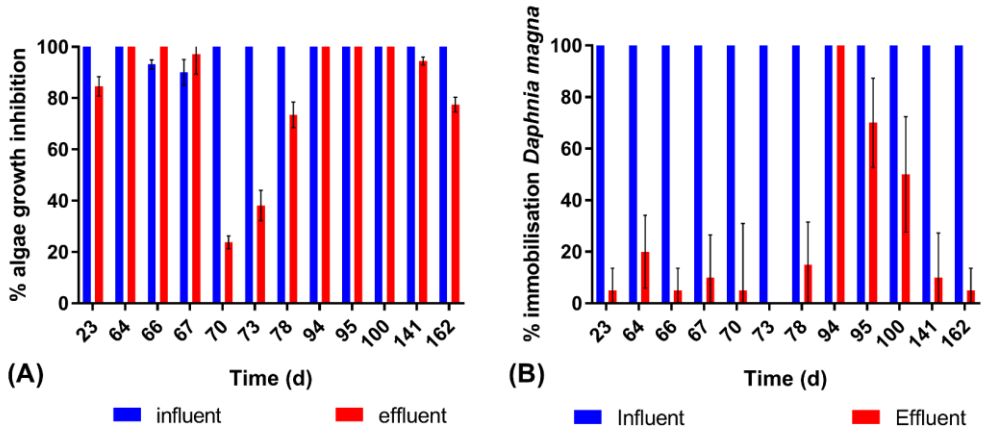

Figure 5: Effluent and influent ecotoxicity of different samples during the whole reactor operation tested with (A) *Pseudokirchneriella subcapitata* and (B) *Daphnia magna*.