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Abstract

Research has demonstrated that the feeding pattern of synthetic wastewater plays an important role in sludge characteristics during biological wastewater treatment. Although considerable research has been devoted to synthetic wastewater, less attention has been paid to industrial wastewater. In this research, 3 different feeding strategies were applied during the treatment of tank truck cleaning (TTC) water. This industry produces highly variable wastewaters that are often loaded with hazardous chemicals, what makes them challenging to treat with activated sludge (AS). In this study it is shown that the feeding pattern has a significant influence on the settling characteristics. Pulse feeding resulted in AS with a sludge volume index (SVI) of 68 $mL.gMLSS^{-1}$ ± 15 $mL.gMLSS^{-1}$. Slowly and continuously fed AS had to contend with unstable SVI values that fluctuated between 100 and 600 $mL.gMLSS^{-1}$. These fluctuations were clearly caused by the feeding solution. The obtained settling characteristics are being supported by the microscopic analysis, which revealed a clear floc structure for the pulse fed AS. Ecotoxicological effluent assessment with bacteria, Crustacea and algae identified algae as the most sensitive organism for all effluents from all different reactors. Variable algae growth inhibitions were measured between the different reactors. The chemical and ecotoxicological effluent quality was comparable between the reactors.

Keywords: *Daphnia magna*, ecotoxicity, feeding pattern; *Raphidocelis Subcapitata*, sequencing batch reactor, settling, tank truck cleaning, *Vibrio fischeri*

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1. Introduction

Tank truck cleaning (TTC) companies create a highly variable, complex and ecotoxicologically sensitive wastewater (De Schepper et al. 2010). Due to intermittent cleaning of tank truck interiors, a wide range of transported products (petrochemical products, food products, (hazardous) chemicals) contaminates the cleaning water. Mostly, physico-chemical and biological wastewater treatment techniques are combined to treat TTC wastewaters. Cleanings that contain hazardous compounds challenges the biological treatment because sludge can be inhibited or a bad removal efficiency can occur. Various types of biological wastewater treatment systems exist for the treatment of domestic and industrial wastewater. The two most common systems are the conventional continuous flow activated sludge (CAS) reactor and the sequencing batch reactor (SBR). To avoid inhibition due to a toxic influent composition, most of the biological treatment plants were built in a CAS configuration (Wilderer et al. 2001; USEPA 2000). This type of reactor has been successful for the treatment of various types of wastewater, although the system is well known for sludge settlement limitations due to the presence of filamentous microorganisms (Martins et al. 2004). In order to suppress growth of filamentous microorganisms, high substrate concentration gradients are recommended in the reactor, as is the case in SBRs (Chudoba et al. 1973, 1985).

Biological wastewater treatment can be successful if activated sludge is able to degrade the polluting components. This biodegradation ability depends on the adaptation of activated sludge to the wastewater that has to be treated. The removal efficiency of difficult biodegradable chemicals like organonitriles (Li et al. 2007), elements which are present in dairy wastewaters (Palela et al. 2010), MDEA (N-methyl-diethanolamine) (Pitas et al. 2012), 4-methylaniline (Moreno-Andrade et al. 2012), and phenol and cyanide (Papadimitriou et al. 2009) significantly improved after adaptation of the sludge. The previously cited studies all mentioned the necessity of microbial adaptation during the biological treatment. Four of these studies investigated the adaptation in SBRs. Papadimitriou et al. (2009) made the comparison between adaptation in a SBR and a CAS for phenol and cyanide removal. This study showed the highest pollutants removal capacity and thereby the lowest effluent concentrations in the SBR system. Effluent chemical oxygen demand (COD) was lower than 400 mg $O_2.L^{-1}$ in the SBR and higher than 600 mg $O_2.L^{-1}$ in the CAS. Besides the effluent quality, also sludge volume index (45 $mL.g^{-1}$ in SBR and 100-150 $mL.g^{-1}$ in CAS) was superior in the SBR.

During biological treatment sludge settling characteristics are important for the operation of the treatment plant. Different studies designated the feeding time as an important parameter, which influences the settling, e.g. Ciggin et al. (2011); Cubas et al. (2011); Guo et al. (2014); Houtmeyers et al. (1980); Martins et al (2003) and Verachtert et al. (1980), all of whom investigated the settling characteristics of activated sludge fed with synthetic wastewater. In all these studies it was shown that a short feeding time, between 1 and 10 minutes, resulted in good settling sludge. The corresponding SVI in all studies was lower than 150 $mL.gMLSS^{-1}$ for the rapidly fed reactors and above 300 $mL.gMLSS^{-1}$ for the slowly fed reactors.

Guo (2014) investigated long-term effects of various carbon sources (methanol, ethanol, propionate, acetate, glucose and starch) and feeding patterns on sludge characteristics and the growth of filamentous bacteria. The carbon sources were initially fed in a feeding period of 10 min, after which the feeding time was increased to 180 min. During the first period (with a feeding time of 10 minutes), SVI for all the components was stable between 100 and 200 $mL.g^{-1}$. After changing the feeding time, SVI varied between 300 $mL.g^{-1}$ and 800 $mL.g^{-1}$. The study of Cubas (2011) used bactopeptone as substrate and focussed mostly on the effluent quality. The feeding was done in 1 minute, 1 hour or 4 hours. As a result of the longer feeding time, effluent total suspended solids increased by over 100% and effluent COD increased from 50 $mgO_2.L^{-1}$ to 110 $mgO_2.L^{-1}$. Dockhorn (2001) showed that the effluent COD removal efficiency for domestic wastewater improved when mixing conditions approached pulse feeding. At lower sludge retention times and low temperatures, the SBR showed a higher process stability compared to the continuous flow plant.
Van den Eynde (1982) investigated the relation between the substrate feeding pattern and the settling characteristics during the treatment of industrial wastewater, as one of few. Three different wastewaters were selected for this study: brewery, dairy and petro-chemical wastewater. The previous hypothesis that intermittent systems with short feeding times were dominated by floc forming bacteria with good settling characteristics was confirmed in this study.

Water quality standards and discharge limits are intensively strengthen worldwide last years. To predict the ecotoxicological effect of a complex effluent for the receiving aquatic ecosystem, whole effluent toxicity (WET)

testing is recommended by different government agencies. Different studies investigated the effluent toxicity of various water samples with a battery of bacteria, Crustacea and algae to measure acute toxicity, e.g. Liu et al. (2002); Mendonça et al. (2013); Ra et al. (2007); Reginatto et al. (2009) and Rosa et al. (2010). All these studies made a comparison between different test organisms. Papadimitriou (2009) is the only study in literature that also compares the ecotoxicological quality of the effluents with a different feeding time. The effluents of the SBR had an inhibition of 10% for the test organism *Vibrio fischeri* compared to 70% inhibition for the effluent of the CAS. TTC wastewater is known as a critical industry for the production of harmful ecotoxicological water. It is demonstrated that no correlation between the chemical effluent quality and ecotoxicological quality can be observed (Dries et al. 2013, 2014; Huybrechts et al. 2014; De Schepper et al. 2010).

The aim of the present study was to investigate the importance of the feeding pattern during the biological treatment of different tank truck cleaning wastewaters. Sludge was subjected to a very short 2 minutes pulse feeding, a 21 hours feeding period and a 24 hours continuous feeding. The effect on sludge characteristics like settling rate, SVI and microscopic structure was investigated. The influence on the chemical effluent quality was observed in terms of COD and dissolved organic carbon (DOC). Lastly toxic influence of the influent on activated sludge performance and activity was investigated as well as the ecotoxicological influence of the effluent on the aquatic environment. A comparison between the different feeding patterns was made to evaluate the impact on the ecotoxicological effluent quality. Different trophic levels of the aquatic ecosystem were tested with acute toxicity tests (bacteria, Crustacea and algae).

2. Materials and methods

2.1 Industrial wastewater and seed sludge

The seed sludge used for the reactors in this study was taken from a TTC company in the harbour region of Antwerp (Belgium). The company is involved in the interior cleaning of tank trucks that transport a wide range of chemicals (dry and wet bulk products). The wastewater treatment plant combines physico-chemical and biological treatment techniques. The seed sludge used to start up the reactors in this research came from the biological treatment step and had a MLSS concentration of 5.94 ± 0.05 $g.L^{-1}$, MLVSS concentration of 5.4 ± 0.07 $g.L^{-1}$ a sludge volume (SV) of 980 mL after 30 minutes settling, SVI of 165 $mL.g^{-1}$ and a settling velocity of 0.01 $m.h^{-1}$. Due to this poor settling characteristics, a dissolved air flotation (DAF) is used to separate sludge and effluent in the industrial installation. A final treatment of the effluent happens with a sand filtration to remove suspended solids.

Wastewater composition was variable during the experimental period and is shown in table 1. Seven different influent batches were collected at the TTC company and treated consecutively in the lab. Each influent was taken after a chemical flocculation coagulation pre-treatment unit that was installed to remove non soluble organic pollutants. The average total COD and DOC during the whole experimental period were 1521 ± 752 mg $O_2.L^{-1}$ and 473 ± 248 mg $C.L^{-1}$ respectively.

2.2 Laboratory activated sludge systems, setup and operation

Experiments were conducted using three automated lab-scale reactors with an internal diameter of 230 mm. The reactors are referred to as SBR fast feeding (SBR_{FF}), SBR slow feeding (SBR_{SF}) and continuous flow reactor (CFR) with a feeding time of 2 minutes, 21 hours and 24 hours respectively. Hydraulic retention time of the reactors was 4 days from day 0 to 50 and 3.25 days for day 51 to 160. During the first 50 days, 3L of wastewater was added. From day 51 to day 160, 4L of wastewater was added daily to increase the organic loading rate. Due to this volume exchange ratio of the SBRs ranged from 25% to 31%. The feeding volume was increased during the operation with influent batch 3. The effect of this increasing feeding volume was pointed out by separating the results of influent batch 3 in a section “a” (3L influent) and a section “b” (4L influent).

SBR operation of SBR_{FF} and SBR_{SF} involved all the necessary phases for biological carbon removal in 1 cycle, i.e. pre-aeration phase, aerobic feeding, aerobic reaction, excess sludge withdrawal, settling and effluent discharge. The CFR was mixed and aerated continuously and fed 24h a day with a feeding rate of 2.78 $mL.min^{-1}$. The reactor was equipped with an internal settling unit. The different operating times of the SBRs were shown in table 2.

To satisfy the nutrient requirements, nitrogen and phosphorus were added to the wastewater as KNO_3 and K_2HPO_4 to ensure a COD:N:P ratio of 100:5:0.8 (Eckenfelder et al. 2008). Nitrate was used as nitrogen source instead of the commonly used NH_4Cl to avoid nitrification.

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

Different probes were used to collect data from 3 parameters in the reactors, namely oxygen, pH and oxidation reduction potential (ORP). The dissolved oxygen (DO) concentration in the reactors was monitored with a Hach Lange LDO sc sensor, pH and (ORP) were measured with Jumo TecLine sensors. The signals of those sensors were transferred by a process field bus (profibus) from a Hach Lange sc 1000 module to the PLC.

Dissolved oxygen concentration in SBR_{FF} and SBR_{SF} was always controlled between a lower and upper DO set point of 1.5 and 5.0 mg $O_2.L^{-1}$ respectively. pH was measured but not controlled since pH was always between 7.3 and 8.5. The sludge retention time (SRT) was kept on 30 days in all reactors by removing a volume of 400mL (day 0 -50) or 430 mL (day 51 – 160) excess sludge every day. This SRT is the same as in the industrial plant were the seed sludge was originated. Temperature in the reactors was equal to room temperature which was always between 20°C and 25°C.

2.3 Chemical analyses

Different chemical analyses were performed to characterise the used influent wastewater and obtained effluents. Chemical oxygen demand (COD) was measured with Hanna Instruments (HI) (Belgium, Temse) COD Tests HI 93754A-25 Low range and HI 93754B-25 Medium range tubes. Chloride and ammonia concentrations were measured with the HI 3815 and HI 93715-01 test kit from Hannah Instruments. pH and conductivity were measured with the HI 9023 microcomputer pH meter, respectively the HI 9033 multi range conductivity meter. Phosphorus was measured using PhosVer3 powder pillows from Hach Lange (Belgium, Mechelen).

Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume (SV) and sludge volume index (SVI) were measured in accordance with the procedures described in Standard Methods for the Examination of Water and Wastewater (1998). Settling rate, SV and SVI were determined with a 1L graduated cylinder with a height of 34.5cm and an internal diameter of 6.1cm. Measurements were always executed at the end of the aeration step and before the settling phase, so sludge was always in an endogenous state.

Dissolved Organic Carbon (DOC) was analysed during the experiments with a Sievers InnovOx Laboratory Total Organic Carbon Analyser. All samples were filtered before they were analysed using a glass microfiber filter of 1.2µm.

2.4 Image analysis

Endogenous sludge, taken before the settling phase, was used for morphologic investigation. Image characterization of the AS was performed with a Motic BA 310 microscope by using an EF-N Plan 10x0.25 ocular.

2.5 Ecotoxicological analyses

Three different acute toxicity tests were performed on unfiltered influent and effluent samples. The following test were conducted: (a) the 30 min bacterial bioluminescence inhibition test (ISO 11348-3) using *Vibrio fischeri.* A commercial test (with freeze-dried *Vibrio*), namely 1243-500 BioTox™ Kit from Aboatox Oy (Finland) was used. The test protocol involved combining 500 µL of influent/effluent (with adjusted salinity of 2% NaCl) with 500 µL of activated bacteria what makes a dilution of 50%. After a contact time of 30 min at 15°C, the decrease of light intensity was measured with a portable tube luminometer (Berthold Technologies Junior LB 9509). The inhibitory effect is compared to a negative control (2% sodium chloride). Hereby, the percentage inhibition is calculated.

(b) 48 h *Daphnia* immobilization test (OECD, 202) using *Daphnia magna* The water fleas used in the experiments originated from a culture reared in the laboratory for several years. The influent and effluent inhibition experiments were performed in the standard OECD TG202 medium for conducting the acute immobilization test with *Daphnia magna* (OECD guidelines 202, 1992). The medium used 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. The animals were always acclimated to the medium for at least three generations before starting the experiment. During the rearing period, they were fed with a mixture of *P. subcapitata* and *Chlamydomonas reinhardtii* in a 3:1 ratio. Three times a week, the medium was renewed, and *Daphnia magna* were fed with 400.10^6 algal cells/L. The culture was maintained at 21.1°C under a 14/10-h light/dark photoperiod. For all experiments, maximum 24h-old D*aphnias* were used, which were isolated after birth. In total, 5 *Daphnias* were incubated with each sample and every sample was measured in triplicate. 8mL total volume of effluent and feeding medium was preserved for each *Daphnia*. Effluent samples were diluted 10% with feeding medium, influent samples 50%.

(c) the 72 h algal growth inhibition test (OECD, 201) using *Raphidocelis Subcapitata*. The growth medium used in the experiments was modified following the OECD TG201 guidelines for conducting standard algal growth inhibition tests (OECD guidelines TG201, 1992) with 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 $Na_2EDTA.2H_2O$) for at least 24 h before inoculation with algae. Reagent-grade chemicals purchased from VWR International and ultrapure water (resistivity, 18.2 M; Milli-QTM; Millipore) were used for preparation of the media. Algal cultures were maintained at 22°C and illuminated with light 4440-8880 lux (400-700nm) under a 14/10-h

light/dark photoperiod. Algal cultures were incubated in a Snijders scientific and stirred at 100 rpm with a GFL 3012 shaker to provide oxygen.

To maintain exponential growth of algae, enough inoculum to provide an initial cell density of 2.10^7 up to 5.10^7 cells/L was taken each week from a pre-culture and transferred to 25mL of sterile medium. The slant culture was acclimated to the medium for three weeks. The number of cells in the culture was monitored using a Beckman Multisizer Z3 Coulter counter. Algae inhibition measurement occurred in 48 well plates. The outer row of the plate was filled with demineralised water to avoid natural evaporation and dry up of the wells. Each sample was measured in triplicate and blanks in sextuple. Effluent samples of the reactors were measured in a ratio of 90% sample and 10% of dilution water. Influent samples were 50% diluted.

2.6 Modelling tool - EPI Suite™

Based on the cleaning list of the company, individual component toxicity was simulated to approach the ecotoxicological effluent risk of a specific compound. This simulation provided a relative number that makes it possible to compare the risk of the cleaned components relatively. A simulation was made by using experimental ecotox data available in literature and a simulation based on predicted toxicity levels by using EPI Suite™. Estimation Programs Interface (EPI) Suite™ was used as modelling tool to predict ecotoxicity of the cleaned cargo products. EPI Suite™ is Windows®-based software by EPA's and Syracuse Research Corp. EPI Suite™ uses a single input (chemical name, CAS-number or chemical structure) to run the fate models STPWIN™ and ECOSAR™. STP is a sewage-treatment plant model and includes the calculation of biodegradability, sorption and volatilization of an organic component during the wastewater treatment process. ECOSAR is a predictive model that uses the ecological structure activity relationships to estimate the acute (short-term) toxicity and chronic (long-term or delayed) toxicity to aquatic organisms. Data for fish, aquatic invertebrates and aquatic plants are available.

For each common chemical in the influent a relative toxic effect factor was calculated with the following formula (De Schepper et al. 2010):

$$Relative\ risk\ number\ i = \frac{S_i}{EC_{50,i} or\ IC_{50,i}} . f_{i,eff} \quad (1)$$

where S_i ($mg.L^{-1}$) is the solubility of compound i (compound solubility is limited to a maximum of 100 $mg.L^{-1}$ to avoid overestimation of the risk exhibited by highly soluble compounds), $EC_{50,i}$ ($mg.L^{-1}$) is the (predicted) compound toxicity of component i towards algae (72h growth inhibition test), $IC_{50,i}$ ($mg.L^{-1}$) is the (predicted) toxicity towards *Daphnia* (48h immobilisation test) and $f_{i,eff}$ is the predicted residual effluent fraction of the compound after biological treatment as predicted using the STPWIN modelling tool.

When the relative risk number of a cleaned component becomes equal to 1, a significant inhibition of 50% for the tested organism will be observed.

3. Results

3.1 Influent analysis

During the experimental period of 160 days, 7 different influents were fed to the reactors. Results for the chemical and ecotoxicological measurements of the influents are shown in table 1. For all influents, a low amount of suspended COD is measured. The fraction of soluble COD (sCOD) to total COD was always higher than 90%. The average pH, conductivity and chloride concentration were respectively 6.9 ± 0.3, 1.7 $mS.cm^{-1}$ ± 0.5 $mS.cm^{-1}$ and 272.9 ppm ± 54.1 ppm.

Besides the chemical quality, also the ecotoxicological quality of the influent was assessed. All influents were diluted 50%. Results are shown in table 1. For all influents, 100% toxicity for the test organism *Daphnia magna* was measured. For *Vibrio fischeri*, toxicity levels between 62.0% and 99.6% were observed. The lowest negative impact for the test organisms was measured for influent batch 2. Also for the algae, batch 2 had the least negative impact with a growth inhibition of 73.5%. All other influent batches had a growth inhibition of 100%. These results indicate the presence of chemical compounds with a high toxicity in the accepted cleaning cargo of the company for all tested organisms.

3.2 Sludge characteristics

After inoculation of the reactors, different influents with a changing COD concentration were fed (table 1). Due to this a changing food to mass ratio (F/M) was imposed to the reactors (figure 1). During the operation time, average MLVSS concentration in the reactors was 1.7 ± 0.6 gMLVSS.L^{-1}, 1.6 ± 0.7 gMLVSS.L^{-1}, 1.6 ± 0.8 gMLVSS.L^{-1} for SBR_{FF}, SBR_{SF} and CFR respectively. During the first 3 feeding batches (batch 1-3), F/M stayed almost equal in all reactors with a value around 0.11 kgCOD.(kgMLVSS.day)$^{-1}$. The following batches (4-7) resulted in higher F/M ratios with a value between 0.16 and 0.47 kgCOD.(kgMLVSS.day)$^{-1}$. The lower F/M values observed for SBR_{FF} were caused by a higher MLVSS concentration in the reactor during this period.

In all reactors, SVI was monitored regularly. During the first 10 days, almost no difference was observed between the three reactors with a SVI of 200 to 280 mL.$gMLSS^{-1}$ (figure 2). During day 12 and 13 an important improvement from 280 mL.$gMLSS^{-1}$ to 135 mL.$gMLSS^{-1}$ was observed for SBR_{FF} and SBR_{SF}. The CFR showed no distinct improvements during these operation days. After day 20, a stable settling in SBR_{FF} was observed of 68 ± 15 mL.$gMLSS^{-1}$ until day 160 with a sludge settling rate between 3 and 4 m.h^{-1}. The settling characteristics of SBR_{SF} and CFR were clearly influenced by the feeding solution. A new influent batch on day 72 had a strong negative impact on the settling of the sludge. No changes occurred after a new feeding batch on day 93. Influent batch 6 (started at day 108) had a positive effect on the settling during the whole feeding period. Switching to influent batch 7 had again a negative effect on the SVI of the CFR in contrast to the SBR_{SF} were a constant SVI was observed.

The observed results of the settling characteristics were confirmed by the microscopic structure of the sludge which is shown in figure 3. The seed sludge had an irregular structure with open sludge flocs which indicates poor settling sludge. Compact sludge flocs with a diameter of approximately 250µm appeared in SBR_{FF} (figure 3 (a)) after 25 days. During the next experimental days' flocs became darker and denser. The well-formed floc structures indicate the formation of good settling sludge which was also confirmed by the SVI values (figure 2). In SBR_{SF} (figure 3 (b)) and CFR (figure 3 (c)) no well-formed flocs were present. After starting up the reactors, sludge evolved to small and weak open flocs (day 25). After 45 days (day 70) small weak flocs were observed that contained filaments. Gradually this structure evolved to an irregularly shaped floc structure with filamentous organisms (day 100). At the end of the experimental period, sludge evolved again more to open flocs with the presence of filaments. Complete filamentous bulking did not occur in the reactors.

3.3 Chemical effluent quality

During the experimental period of 160 days, 7 different influents were fed to the reactors. Each influent resulted in a different effluent quality which is shown in figure 4. The lowest effluent DOC concentrations were obtained with influent batches 1 and 2. The highest effluent concentrations were retrieved with batch 6. Increasing the feeding volume from 3 to 4L during the treatment of batch 3 had a negative impact on the effluent quality of all three reactors. During the whole period an average removal rate of 77.8% - 96.3% with an average of 89.9% was obtained. Furthermore, no correlation between the influent DOC and effluent DOC could be found.

3.4 Ecotoxicological effluent quality

Effluent toxicity was measured using standard acute toxicity tests with *Vibrio fischeri, Daphnia magna* and *Raphidocelis Subcapitata* which are very well described in literature. Different organisms were used to have an interconnected ecosystem process with a battery that was composed of 3 different phyla (USEPA 1991).

Samples for effluent toxicity analyses were taken for each batch. For all effluents no toxicity was observed with the *Vibrio fisheri* inhibition test (samples 50% diluted). Results for the *Daphnia magna* immobilisation analysis are shown in figure 5a. Daphnia magna immobilisation was lower than 20% in all effluent samples. For SBR_{FF} only 3 batches showed an immobilization effect, which was less than 10%. 5 out of the 7 effluents of SBR_{SF} and the CFR had an immobilisation effect. The highest effect was 15% ± 15% for effluent batch 2 of the CFR.

For all effluents, a significant inhibition of *Raphidocelis Subcapitata* was measured (samples 10% diluted) ranging from 42.4% to 100%. Effluent batch 3 and 4 completely inhibited algae growth, which is shown on figure 5b.

3.5 Theoretical toxicity prediction/estimation

For each component, and each organism (*Daphnia* and algae), 4 relative risk numbers were calculated. Two numbers estimated the ecotoxicological risk of the component before and after (based on the expected removal rate simulation of EPI Suite™) biological treatment. Those two relative risk numbers were calculated with experimental toxicity data of the EC_{50} and simulated concentrations by using EPI Suite™. Experimental toxicity concentrations were obtained from EPI Suite™ and Material Safety Data Sheets (MSDS).

The relative risk number have to be interpreted by comparing the calculate numbers between different components. The higher this number, the more significant the risk for a negative ecotoxicological impact by this component.

The results of the calculated relative risk numbers are shown in figure 6 (algae) and figure 7 (*Daphnia*) for the 20 most cleaned components. The daily cleaning list of the company was analysed to determine the amount of cleanings from each specific component. Cleanings from the day of sampling and 3 days in advance were used to calculate the total amount of cleanings from each component. Due to this, the residence time distribution of the buffer tank can be taken into account. Methylmethacrylate is distilled out of the list as the most harmful component for algae before (relative risk number 386) and after (relative risk number 29.4) treatment with the theoretical simulated data of EPI Suite™. This is in contrast with the experimental data that shows a low relative risk number before (2.7) biological treatment and no risk (0.2) after treatment. For *Daphnia*, butylacrylate can be seen as the most harmful before (relative risk number 32.4) treatment and after (2.25) treatment with the simulated data of EPI Suite™. Although, in the experimental data, no indication for toxicity can be found. In contrast, N-methylpyrolidine shows no toxicity with the simulated data but shows a significant relative risk number before (81.3) and after (6.45) the biological treatment with experimental toxicity levels. As shown in figure 6 and figure 7, biological treatment can reduce toxicity significantly, differences between the components can be observed. Generally higher relative risk numbers for algae were simulated with the experimental data compared to *Daphnia*s. This is in accordance with the obtained experimental data of the ecotoxicity measurements where algae have been seen as the most sensitive organism.

4. Discussion

In this study, 7 different influent batches were fed over a period of 160 days to three reactors with a feeding time of 2 minutes, 21 hours and 24 hours respectively. The objective of the study was to investigate the effect of this feeding time on (i) the sludge characteristics (ii) chemical effluent quality and (iii) ecotoxicological effluent quality. It is clearly shown that the reactor with the short feeding time of 2 minutes resulted in a good settling sludge with an average SVI of 68 $mL.gMLSS^{-1}$ ± 15 $mL.gMLSS^{-1}$. The reactors with a feeding time of 21 and 24 hours had highly variable settling characteristics with predominantly deficient settling properties. The poorest settling rates were obtained during batch 4 and 5, a settling volume of 900 mL after 30 minutes was observed for those reactors. In extension of these results, similar findings were obtained in the industrial treatment plant where a 24 hours feeding period resulted in sludge with poor settling characteristics. The different settling characteristics in the reactors can be explained by the kinetic selection theory of Chudoba (1973, 1985). Due to periods of high and low concentration during pulse operation, another biogenesis will grow than when substrate concentration is always low (Wanner et al. 1992). Problems with filamentous bulking and bad settling sludge still occurs worldwide (Martins et al. 2004). Jenkins et al. (2004) showed that a relation can be found between the F/M ratio and the development of filaments. Filamentous organisms can develop at F/M ratios between 0.05 and 0.8. the applied F/M in this study was in the same range. Methods like chlorination (Guo et al. 2012), ozonation (Cavarelli et al. 2006) and hydrogen peroxide addition (Saayman et al. 1998) are shown to be effective in the suppression of filaments.

Besides the methods discussed above, also operational factors of the biological reactor were investigated to improve sludge characteristics. For example, dissolved oxygen concentration (Martins et al. 2003), temperature (Knoop and Kunst 1998), organic loading rate (Chudoba et al. 1974; Van Dierdonck et al. 2012), nutrient deficiency (Peng et al. 2003) and substrate composition (Ciğgin et al. 2011; Gulez and De Los Reyes 2009; Martins et al. 2011; Van Dierdonck et al. 2013) are shown to be important. In this study, the feeding time of each reactor was kept constant. Influent composition and organic loading rate were variable. This is shown in table 1 (influent composition) and figure 1 (F/M). The short feeding time resulted in a good and stable settling during the whole study which is very important to mention for the operational strategy and efficiency of tank truck cleaning wastewater treatment plants. To the best of our knowledge this is never been described before for TTC wastewater. Similar effects were observed for brewery wastewater, dairy- and petrochemical wastewater in Van den Eynde et al. (1982). They described SVI values around 50 $mL.gMLSS^{-1}$ in the pulse fed reactor and values above 250 $mL.gMLSS^{-1}$ for the continuously fed reactors, for the aforementioned wastewaters. No variations in the feeding solution were tested. The average experimental time of the experiments was 30 days. More recently, Caluwé et al. (2016) presented results from a changing feeding pattern from continuous to pulse feeding during the treatment of petrochemical wastewater. In contrast to Van den Eynde et al. (1982), 13 different batches were pulse fed over a period 450 days without negatively influencing the settling characteristics which was also observed in this study.

The observed morphology of the activated sludge supported the data from the settling characteristics of the sludge. The good settling sludge of SBR_{FF} showed the formation of small granules while the two reactors with the slow feeding pattern demonstrated an irregular open floc structure with filaments. In the SBR_{FF}, flocs with a diameter over 200µm were observed using microscopy, which is the lower limit to consider it as aerobic granular sludge (Adav et al. 2008). Research already mentioned a relationship between settling characteristics and the morphology of the sludge (Govoreanu et al. 2003; Li et al. 2007 and Van Dierdonck et al. 2013). Although no mathematical correlation was made in this research, it was seen that the appearance of open flocs and filaments was followed by poor settling characteristics. Important to mention is the adaptation time of the activated sludge to the pulse feeding. In 15 days (which is half of the sludge retention time) a significant improvement was obtained of the sludge settling. The same transition period was seen with petrochemical wastewater as shown in Caluwé et al. (2016). Although it is significantly faster than reported by others where 2 to 3 times the sludge age is most common (Van den Broeck et al. 2008). The goal of this study was to monitor the influence of different feeding patterns on the sludge characteristics and effluent quality. Nevertheless, the authors mention that future work can be done to identify the different types of bacteria that were present in the activated sludge in the different reactors. Different staining and identification methods for identification are available. This information could deliver insights and explanations for the measured sludge characteristics and reactor performances.

Chemical effluent quality analyses showed variable effluent DOC values for all reactors, which were influenced by the influent batch used. Earlier research from Dockhorn (2001) demonstrated better reactor performance with a short feeding time during the treatment of domestic wastewater. Cassidy et al. (2000) described better removal of diesel fuel in a pulse fed SBR. Although those studies showed a higher performance during pulse feeding, this was not the case in this study. Equal effluent reactor removal efficiencies were obtained. An explanation for that can be found in the complexity of the wastewater and the presence of chemicals that were not biological degradable. None of the feeding patterns was superior for the development of microorganisms to handle these residual substrates.

The necessity of using a battery of rapid bioassays during ecotoxicological effluent assessment is already discussed in literature (De Schepper et al. 2010; Dries et al. 2013; Dries et al. 2014; Gartiser et al. 2009, Gartiser et al. 2010) and is clearly confirmed by this study. The studied TTC influent and effluent were extremely toxic for *Raphidocelis Subcapitata* while being only moderately toxic towards *Daphnia Magna* and not toxic towards *Vibrio fischeri*. It was not possible to make a correlation between the effluent chemical quality and ecotoxicological quality. The ecotoxicological effluent quality of the SBR_{FF} was comparable to the SBR_{SF} and the CFR. Although, none of the systems was able to remove all eco toxicity during the biological treatment, which was also presented in Dries et al. (2013). The tank cleaning sector is known to produce environmentally hazardous wastewaters which explains the exceptional ecotoxicity measured in this study.

Simulated ecotoxicity data from EPI Suite^{TM}, based on the chemical structure, is not correct and differ significant from experimental data. Furthermore, it is shown that it is impossible to predict ecotoxicity of this type of wastewater by model simulation. Interactions between components cannot be predicted with aforementioned models which makes it impossible to assess effluent toxicity by model simulation.

To conclude, these results suggest that pulse fed activated sludge (SBR_{FF}) can significantly improve the operational conditions of the industrial wastewater treatment plant. Higher settling rates were obtained and no negative impact on the chemical effluent quality is observed. The industrial continuous wastewater treatment plant struggles with poor settling sludge. Changing the system to a pulse fed activated sludge plant would significantly improve those settling characteristics. The assumption can be made that for less variable industrial wastewaters and domestic wastewater, the positive effect of pulse feeding on the sludge characteristics would even be more significant. Especially when the results in this study are compared to the once where synthetic wastewater was used, significant advances for the pulse fed activated sludge can be expected.

5. Conclusion

The present study with industrial tank truck cleaning wastewater showed the importance of the feeding time during the biological wastewater treatment. Good settling characteristics (average SVI of 68mL.gMLSS^{-1}) were obtained with a well-defined morphological floc structures when a short feeding time was applied. The settling properties of the slowly and continuously fed activated sludge was unstable and was influenced by the variable influent characteristics (SVI varied between 70 and 600 mL.gMLSS^{-1}). Depending on the influent batch, a different chemical and ecotoxicological effluent quality was obtained. The effluent DOC varied between 20 and 45 mg.L^{-1}. The feeding pattern had no effect on the chemical nor the ecotoxicological effluent quality. The *Vibrio fischeri* bioluminescence inhibition test showed no toxicity. For *Daphnia magna,* immobilisation was lower than 20% for all measured samples. *Raphidocelis subcapitata* growth inhibitions between 40 and 100% were measured. Using a model, effluent toxicity against algae was predicted, based on individual chemical compound toxicity. It was, however, not possible to predict the toxicity of the total effluent using this modelling approach.

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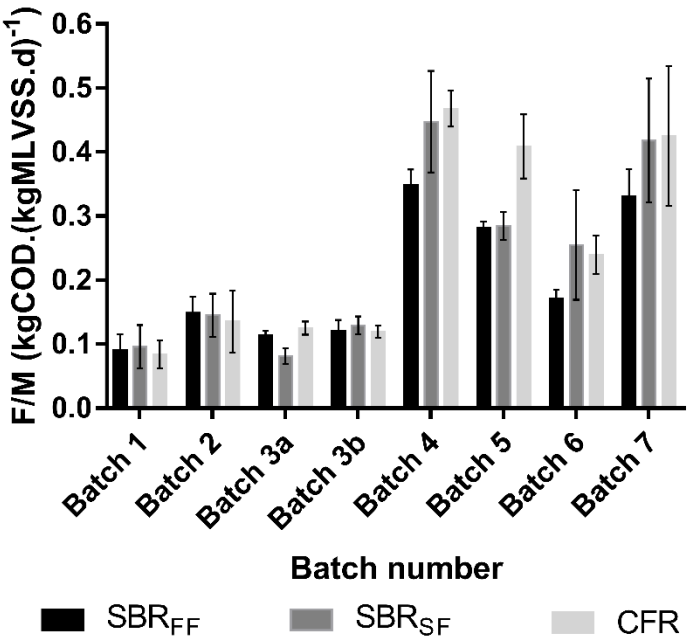

Fig. 1 Average food to mass ratio for all different influents during the whole operation time in the three reactors. Batch 1-3a represents a feeding volume of 3L and batch 3b-7 a volume of 4L. Error bars represent the standard deviation

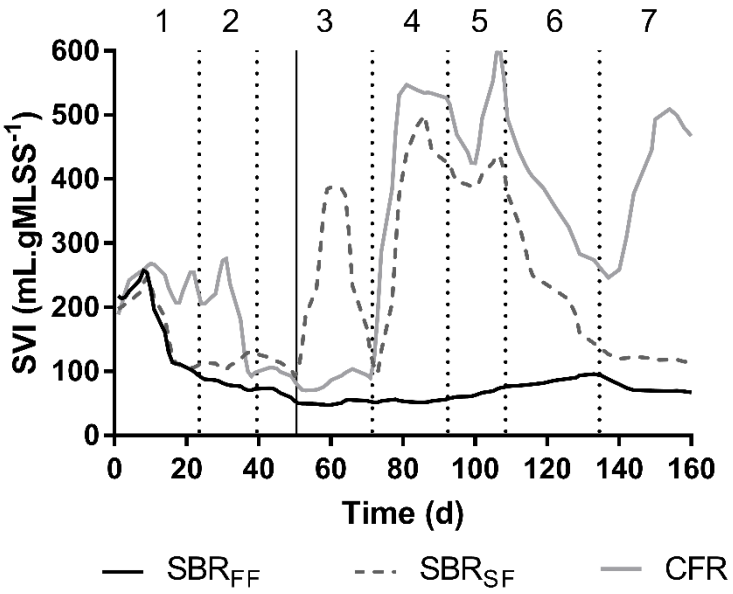

Fig. 2 Evolution of the SVI in the three SBRs. A dotted vertical line represents a new influent feeding. The vertical line on day 50 represents a changing feeding volume from 3 to 4L.

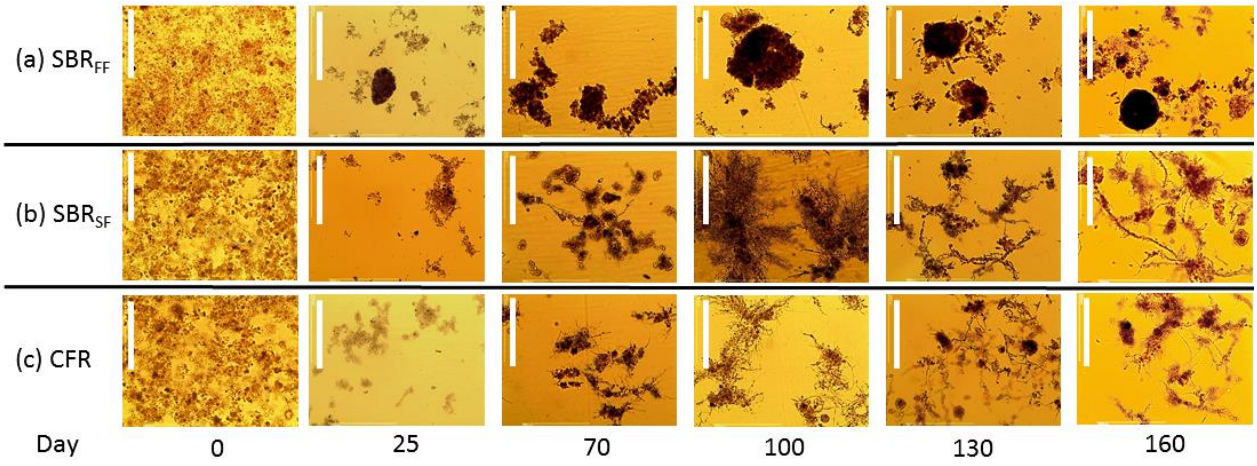

Fig. 3 Microscopic structure of the sludge in the different reactors row (a) $SBR_{FF,}$ (b) SBR_{SF} and (c) CFR. The sampling day is indicated below each column. White lines represent a scale bar of 500µm.

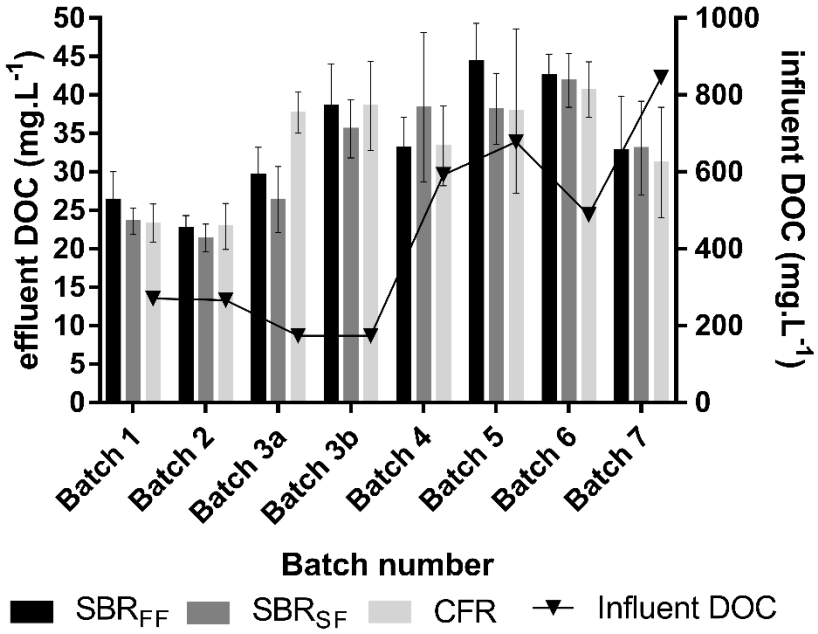

Fig. 4 Average effluent DOC from all three reactors for every different influent batch. Batch 1-3a represents a feeding volume of 3L and batch 3b-7 a volume of 4L. Error bars represent the standard deviation.

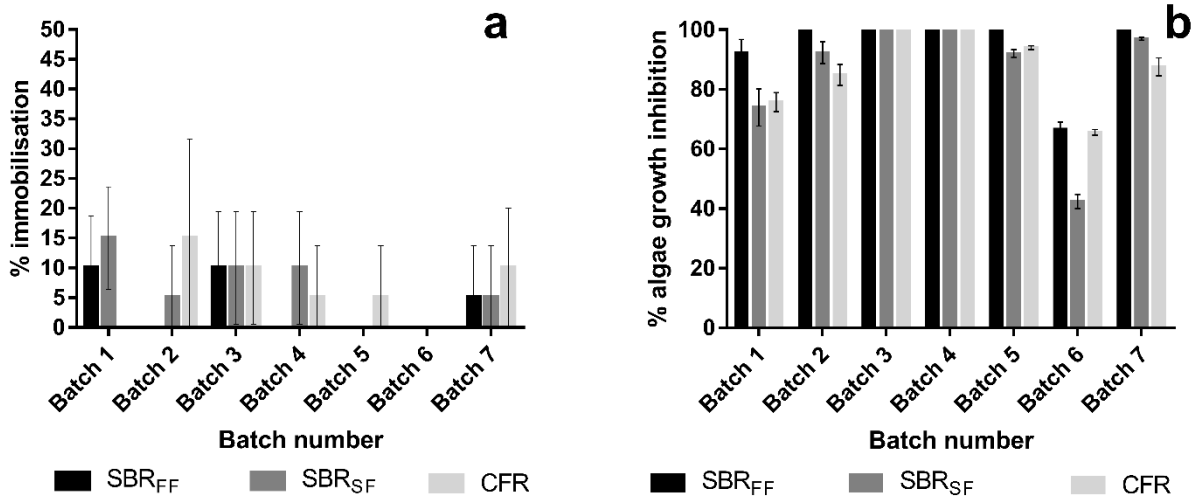

Fig. 5 (a) % immobilisation of *Daphnia Magna* for the different effluents (b) % *Raphidocelis Subcapitata* growth inhibition for all different effluents. Note that both figures have a different scale on the y-axis. Error bars represent the standard deviation.

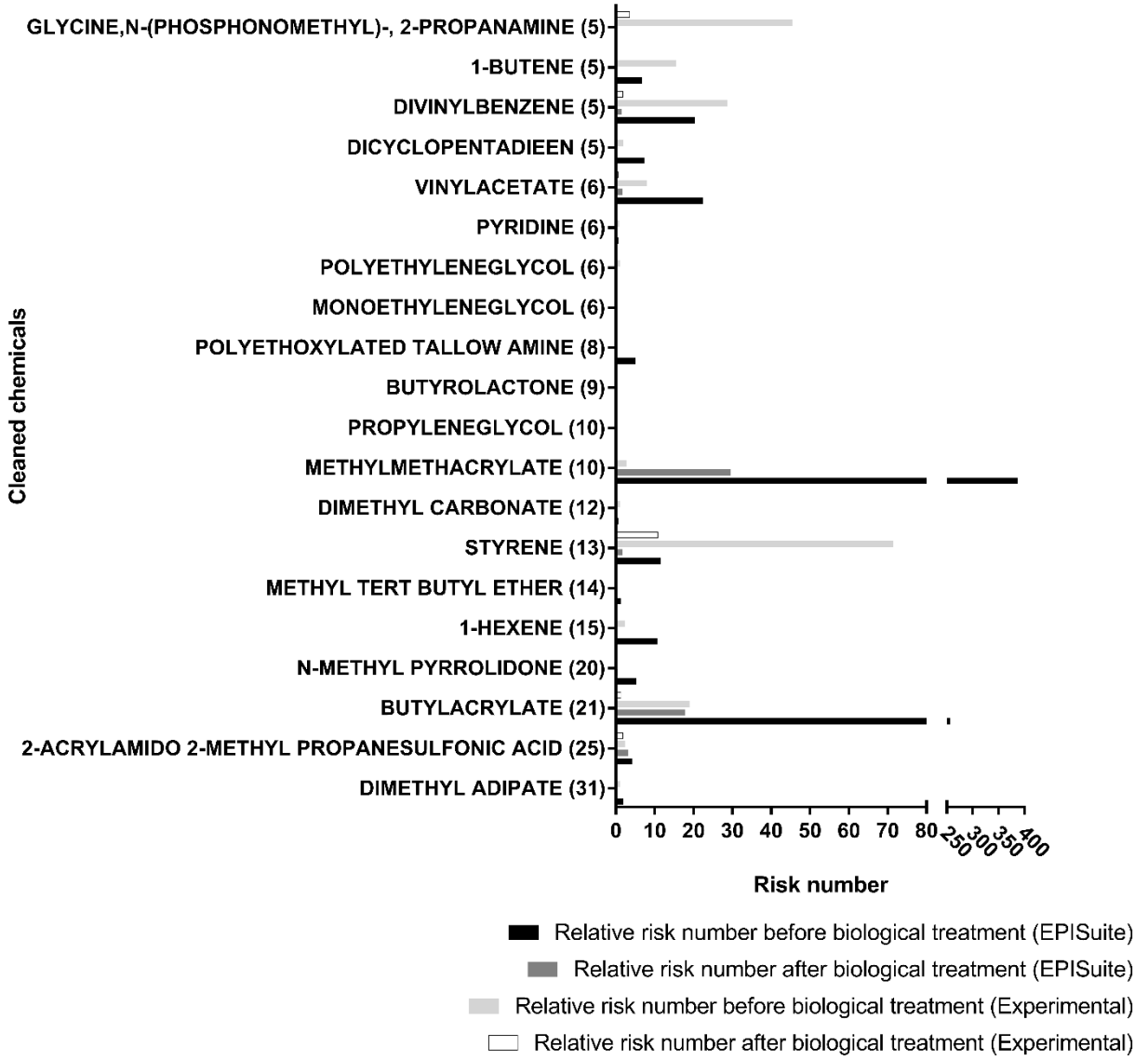

Fig. 6 Relative risk number for algae before and after biological treatment obtained by EPI Suite™ and experimental data for the 20 most cleaned cargo products. The amount of cleanings is indicated between brackets after the component.

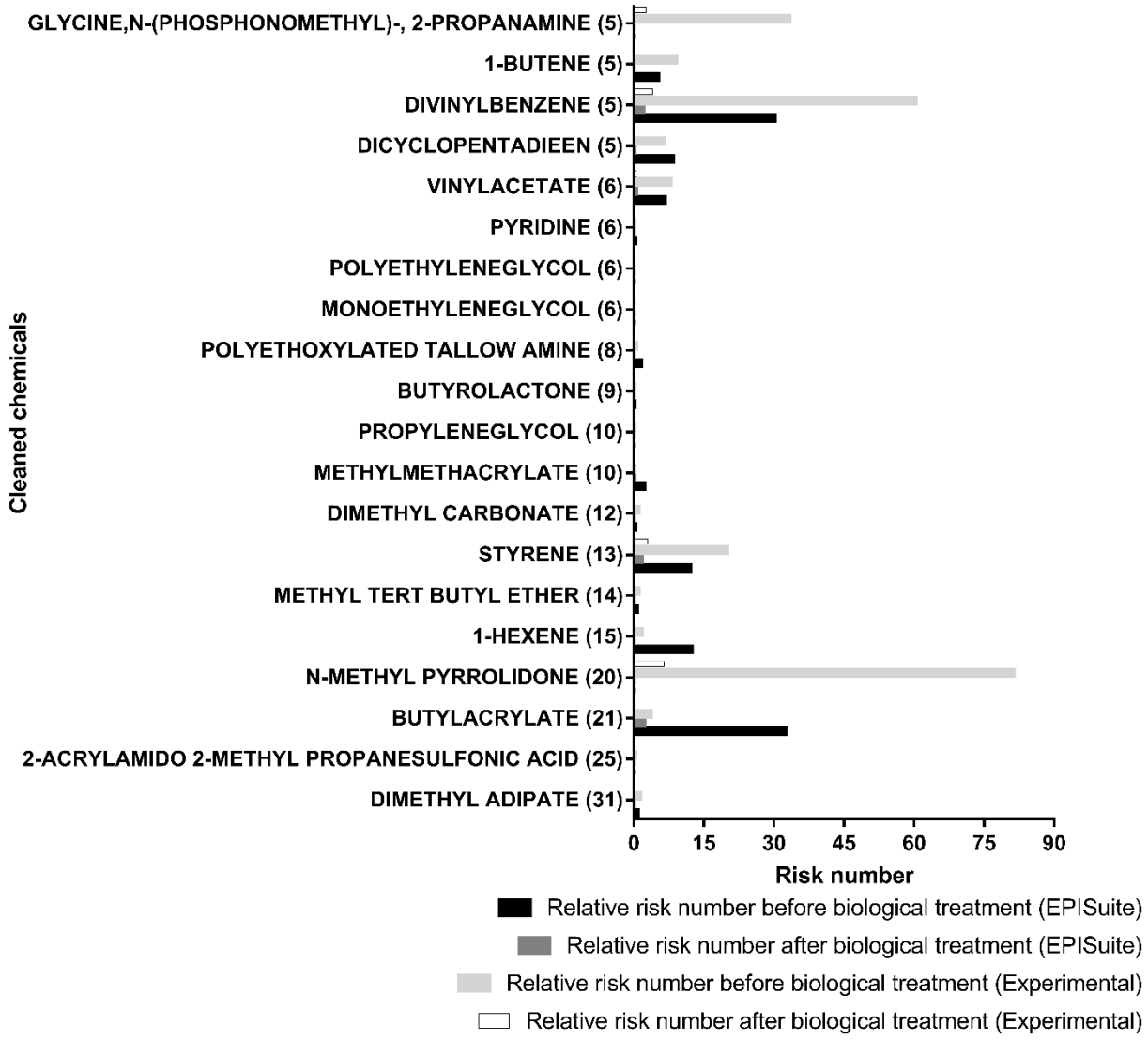

Fig. 7 Relative risk number for *Daphnia* before and after biological treatment obtained by EPI Suite™ and experimental data for the 20 most cleaned cargo products. The amount of cleanings is indicated between brackets after the component.

List of table names

Table 1. Chemical composition and ecotoxicological quality of the different influents. Operation day indicates the days on which the influent batch was used. COD, sCOD and DOC of the influent were measured weekly. pH, conductivity, chloride and ammonia concentration were measured once. (standard deviations are included in the table)

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
Batch used during operation day	1-23	24-39	40-71	72-92	93 -108	109 - 134	135 - 160
COD (mg O_2.L^{-1})	1120 ± 20	960 ± 35	620 ± 18	2502 ± 76	2268 ± 83	1052 ± 43	2122 ± 58

sCOD ($mgO_2.L^{-1}$)	1034 ± 14	941 ± 32	590 ± 27	2436 ± 59	2124 ± 64	964 ± 35	1986 ± 45
DOC (ppm)	270 ± 12	266 ± 9	174 ± 13	592 ± 23	678 ± 26	488 ± 15	845 ± 24
pH	6.89	7.40	6.89	7.08	6.84	6.6	6.57
Conductivity ($mS.cm^{-1}$)	1.6	1.6	1.6	1.1	1.5	1.6	2.7
Cl^- (ppm)	350	220	300	250	270	200	320
NH_4-N (ppm)	10	8.1	7.8	10.0	10.0	8.2	25.6
Bacteria (% bioluminescence inhibition)	77.7 ± 0.3	62.0 ± 0.4	78.0 ± 0.1	98.5 ± 0.01	97.1 ± 0.1	99.6 ± 0.02	99.4 ± 0.01
Crustacea (% immobilisation)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Algae (% growth inhibition)	100 ± 0	73.5 ± 5.0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0

Table 2. Operating conditions of the SBRs

Phase (duration – min)	**SBR_{FF}**	**SBR_{SF}**
Pre aeration	30	30
Aerobic feeding	2	1260
Aerobic reaction	1284	26
Excess sludge withdrawal	2	2
Settling	120	120
Effluent discharge	2	2
Total time	1 day	1 day