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Relationships between chemical oxygen demand (COD) components and toxicity in a sequential anaerobic baffled reactor/aerobic completely stirred reactor system treating Kemicetine

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ABSTRACT

In this study the interactions between toxicity removals and Kemicetine, COD removals, intermediate products of Kemicetine and COD components (CODs originating from slowly degradable organics, readily degradable organics, inert microbial products and from the inert compounds) were investigated in a sequential anaerobic baffled reactor (ABR)/aerobic completely stirred tank reactor (CSTR) system with a real pharmaceutical wastewater. The total COD and Kemicetine removal efficiencies were 98% and 100%, respectively, in the sequential ABR/CSTR systems. 2-Amino-1 (p-nitrophenil)-1,3 propanediol, l-p-amino phenyl, p-amino phenol and phenol were detected in the ABR as the main readily degradable inter-metabolites. In the anaerobic ABR reactor, the Kemicetin was converted to corresponding inter-metabolites and a substantial part of the COD was removed. In the aerobic CSTR reactor the inter-metabolites produced in the anaerobic reactor were completely removed and the COD remaining from the anerobic reactor was biodegraded. It was found that the COD originating from the readily degradable organics did not limit the anaerobic degradation process, while the CODs originating from the slowly degradable organics and from the inert microbial products significantly decreased the anaerobic ABR reactor performance. The acute toxicity test results indicated that the toxicity decreased from the influent to the effluent of the aerobic CSTR reactor. The ANOVA test statistics showed that there was a strong linear correlation between acute toxicity, CODs originating from the slowly degradable organics and inert microbial products. A weak correlation between acute toxicity and CODs originating from the inert compounds was detected.

1. Introduction

Among all the other pharmaceutical drugs and substances, antibiotics are important compounds since they pose a threat to the environment [1]. Antibiotics are present in the effluent from treatment plants and receiving bodies due to treatability difficulties with conventional treatment systems such as aerobic activated sludge system and anaerobic process. For that reason, they spoil the ecological balance, becoming toxic to organisms in the ecosystem [2–7]. Discharges from hospitals and pharmaceutical plants have been shown to cause an increase in bacterial populations resistant to certain antibiotics [8,9]. Antibiotics exhibit toxicity towards certain species in the receiving bodies [10–12]. Toxic effects of common antibiotics on different organisms (bacteria, algae, *Artemia salina*, *Daphnia magna*, etc.) have been found even at very low exposure times [13–16].

Conventional technologies used in wastewater treatment systems do not completely remove the pharmaceutical residues which are then released, via treated effluent, to the environment. Antibiotic residues have been frequently detected in rivers and lakes that receive sewage and industrial effluents and in drinking water systems supplied by those surface waters. Aerobic activated and sewage treatment systems are not equipped for antibiotic removal. Currently, there are no municipal sewage and pharmaceutical activated sludge treatment plants that are engineered specifically for antibiotic removal. The recalcitrance of antibiotics may play an important role in decreasing the COD removal efficiency in affected treatment systems. Substantial parts of the COD and antibiotics may be biodegradable while other parts of the COD may be inert in pharmaceutical wastewaters [17–19]. Effective removal of antibiotics from the treatment plants varies considerably, based on the type of chemical and on the individual biological treatment facilities. Recent papers have demonstrated that pharmaceutical

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wastewaters are biodegradable under aerobic conditions [6,20–22]. Some researchers reported that anaerobic treatment systems could also be used to treat the antibiotics [23–27]. Advanced water treatment technologies can remove many contaminants; however, this technology is expensive and may not be affordable for many treatment plants. Biological treatment methods are preferred to physico-chemical methods for safe disposal, due to their cost effectiveness and efficient treatability of antibiotics.

Not much is known about the toxicity of antibiotics in wastewater treatment plants. It was found that the results of acute toxicity on *Phadectylum tricornutum* exhibited toxicity in a cefalozin antibiotic formulation wastewater [28]. The procaine penicillin G formulation wastewaters have demonstrated an inhibitory effect on *D. magna* in an acute toxicity test [29].

One of the high rate anaerobic reactors is the anaerobic baffled reactor (ABR) and it is resistant to toxic and refractory organics. The ABR is a simple rectangular tank and it is divided into four or five equal compartments [30]. The ABR consists of a series of vertical baffles. The liquid flow is alternately upward and downward between the partitions, and on its upward passage the waste flows through an anaerobic sludge blanket, of which there are four or five. Hence, the waste is in intimate contact with active biomass, leading to higher treatment efficiency. Bacteria within the reactor settle because of gas production and flow characteristics [30].

The anaerobic baffled reactor has several advantages over other anaerobic systems: it has a simple design since no special gas separation, no packing material, no moving parts and no mechanical mixing are required [31]. The unique structure of the reactor causes the partial separation of acidogenesis and methanogenesis. These phases bring about an increase in protection against toxic materials and higher resistance to changes in environmental parameters such as pH, temperature and organic loading [32]. The anaerobic baffled reactor has not been extensively used in the treatment of antibiotic wastewaters. There have been several studies performed with ABR treating different wastewaters, such as whisky distillery wastewater [33], textile dye wastewater [34], decolorisation of dyes [35], wastewaters containing nitrogen [36], swine wastewater [37], domestic wastewater [38], pulp and paper mill [39], palm oil mill effluent wastewater [39] and ice-cream wastewater [40].

The literature survey indicated that no experimental studies have been done investigating the treatability, toxicity and COD subcategories of Kemicetine ($C_{11}H_{12}C_{l2}N_2O_5$) in a high rate compartmentalized anaerobic ABR reactor. Kemicetine is effective against a wide variety of microorganisms, and it is still very widely used in low income countries such as Turkey because it is exceptionally cheap. The risks posed by Kemicetine to aquatic organisms are not very well known. For example, the effects of Kemicetine on anaerobic methanogenic bacteria are unknown. Furthermore, the reason for low COD removals in pharmaceutical wastewater treatment plant has not been thoroughly investigated. The soluble COD parameter alone used for substrate utilization cannot give enough information about the degradation of the organic matter. Therefore, biological degradation parts and inert fraction of COD must be determined since all design calculations need to deal with biodegradable COD. This is important for refractory and toxic wastewaters such as the pharmaceutical industry. The CODs originating from the soluble inert compounds and from the microbial products must be determined for discharge standards since they do not give any reaction in the activated sludge system and are released together with wastewater discharge. However, the CODs originating from the readily degradable COD, slowly degradable COD and fractions that leave the treatment system must be determined in order to assess the biodegradability of the sequential anaerobic ABR/aerobic CSTR reactor system. High antibiotic concentrations, CODs originating from the inert compounds, from the bacterial products and from the slowly degradable compounds in pharmaceutical wastewater may reduce the performance of treatment plants. Therefore, pharmaceutical wastewater containing Kemicetine was processed in a high rate anaerobic ABR reactor in order to investigate the relationships between COD subcategories, intermediate products, and toxicity of the subjected wastewater.

The major objectives of this research can be summarised as follows:

1. To investigate the removal efficiencies of COD and Kemicetine, VFA production, total and methane gas productions of real wastewater containing Kemicetine at different doses in sequential ABR/completely stirred tank reactor (CSTR) systems.
2. To characterise the wastewater composition based on COD originating from slowly, inert and readily biodegradable organic substances and to determine the metabolites of Kemicetine.
3. To monitor the acute toxicity of real pharmaceutical wastewater containing Kemicetine based on *D. magna*, *Photobacterium phosphoreum* and *Chlorella* tests.
4. To detect the relationships between toxicity, intermediate products and COD components.

2. Materials and methods

2.1. Configuration of ABR and CSTR reactors

In this study, a continuously fed ABR connected to an aerobic CSTR was used. The effluent of the ABR was used as the influent of the aerobic CSTR reactor. The ABR can be described as a series of upflow anaerobic sludge blanked reactors (UASB) to forces the wastewater to flow from inlet to outlet [35]. The design characteristic of the ABR permits separation of more sensitive anaerobic populations such as methanogens. The separation of acedogenic and methanogenic phases results in an increase in protection against toxic materials and causes higher resistance to changes in environmental parameters such as pH, temperature and organic loading [30,35]. The ABR is a high rate granulated anaerobic sludge reactor. During upflow, the wastewater is in contact with the active biomass. A simple design, non-mechanistic mixing, inexpensive construction, low capital and operational costs, low sludge generation, high solid retention times and short hydraulic retention times are the advantages of the ABR reactor.

The ABR reactor used in this study was rectangular, box-shaped and with the following dimensions: 20 cm wide, 60 cm long and 40 cm high. The ABR reactor with the active reactor volume (38.4 l) was divided into four equal compartments by vertical baffles. Only three compartments were used throughout this study (effective volume = 28.8 l). The last compartment was used as a settling tank. Each compartment was further divided into two by slanted edge (45 °C) baffles to encourage mixing within each compartment. Therefore, down-comer and up-comer regions were created. The liquid flow was alternatively upwards and downwards between compartment partitions. This provided effective mixing and contact between the wastewater and biomass at the base of each upcomer. In other words, during upflow, the waste flow was in contact with the active biomass and it was retained within the reactor providing a homogenous distribution of wastewater. Additional mixing was not applied to the compartments of the reactor. The width of the downcomer was 4 cm and the width of the up-comer was 11 cm. The passage of the liquid from each compartment to the other was through an opening, 40 mm × 10 mm, which was located about 80 mm from the top of each compartment. The liquid sampling ports were located at a distance of 40 mm from the effluent opening of each compartment. The sludge sampling ports were also located in the center of the compartments, 80 mm from the bottom of each compartment. The influent feed was pumped using a peri-

Table 1
Composition of raw pharmaceutical wastewater containing Kemicetine.

Parameter	Concentration (mg/l)
COD	2800–3300
BOD5	420–627
BOD5/COD ratio	0.15–0.19
TSS	3400–4100
VSS	1400–1900
Chlorides	250–370
Sulfates	120–150
Phospates	15–20
T. alkalinity	1650–1900
TKN	98–135
pH	6.99–7.59
Kemicetine concentrations (in raw wastewater)	32, 49, 125

staltic pump. The outlet of the ABR was connected to a glass U-tube to control the level of wastewater. The produced gas was collected via a porthole in the top of the reactor. The operating temperature of the reactor was maintained at a constant of $37 \pm 1\,^{\circ}C$ by placing the ABR reactor on a heater. A digital temperature probe located in the middle part of the second compartment provided the constant operation temperature. This provided a homogenous temperature in all the compartments of the ABR reactor. The dissolved oxygen concentration was around zero in this reactor.

The CSTR reactor consisted of an aerobic (effective volume = 9 l) and a settling compartment (effective volume = 1.32 l). This reactor was continuously fed from the bottom by a feeding pump with the raw wastewater. The aerobic reactor was aerated by an air pump and porous diffusers to maintain the DO concentrations between 4 and 6 mg/l. The effluent wastewater from the aeration tank to the sedimentation tank passed through holes in a plate inclined at 45° to the horizontal axis. Effluent leaving the sedimentation tank was collected in an effluent tank.

2.2. Seed sludge

Partially granulated anaerobic sludge was obtained from the methanogenic reactor of Pakmaya Yeast Industry and was used as seed in the ABR. Activated sludge was obtained from the aeration tank of the same factory and was used as seed in the aerobic CSTR reactor. The ABR was fed with real wastewater containing Kemicetine. The average total suspended solids (TSS) in the ABR was determined as 54,400 mg/l, while the mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concentration in the CSTR varied between 3500 and 4500 mg/l and between 2950 and 3700 mg/l, respectively.

2.3. Composition of raw wastewater

Antibiotic wastewater containing Kemicetine was obtained from a pharmaceutical industry factory located in Gebze-Istanbul, Turkey. The COD concentration was approximately 3000 ± 450 mg/l. A certain amount of stock Vanderbilt basal medium was added to the raw pharmaceutical wastewater. Vanderbilt mineral medium was prepared in distilled water by dissolving per liter 0.4 g $MgSO_4$, 0.4 g NH_4Cl, 0.4 g KCl, 0.3 g Na_2S, 0.08 g $(NH_4)_2HPO_4$, 0.05 g $CaCl_2$, 0.04 $FeCl_2$, 0.01 g $CoCl_2$, 0.01 g KI, 0.01 g $Na(PO_3)$, 0.5 mg $AlCl_3$, 0.5 mg $MnCl_2$, 0.5 mg $CuCl_2$, 0.5 mg $ZnCl_2$, 0.5 mg NH_4VO_3, 0.5 mg $NaMoO_4$, 0.5 mg H_3BO_3, 0.5 mg $NiCl_2$, 0.5 mg NaWO4, 0.5 mg Na_2SeO, and 0.01 g cystein [41]. This medium was used since it contained all the minerals and the heavy metals which are necessary for the growth of methanogens. 0.5 mg/l of sodium thioglycollate was used to reduce the redox potential of the raw wastewater and thus maintain the anaerobic conditions in the anaerobic ABR reactor. To provide suitable alkalinity and neutral pH, 4000 mg/l $NaHCO_3$ was added to the ABR reactor. The composition of the raw pharmaceutical wastewater containing Kemicetine is illustrated in Table 1.

2.4. Operations of the ABR and CSTR systems

2.4.1. Start-up of the ABR reactor

The adaptation period is very important since the bacterial population used as seed is exposed to the Kemicetine in an anaerobic environment in the ABR reactor. In order to acclimatize the partially granulated anaerobic biomass in the ABR reactor, the anaerobic reactors were operated with synthetic wastewater through 30 days without Kemicetine to reach steady-state conditions. A steady state was arbitrarily considered as a variation of COD in the effluent and variations of methane gas production of less than 5% in 7 consecutive days. The synthetic wastewater was composed of 3000 ± 45 mg/l glucose-COD, 1 mg/l of NH_4Cl and 0.3 mg/l K_2HPO_4 and Vanderbilt mineral medium in order to maintain a ratio of 100:3:1 for BOD/N/P throughout anaerobic treatment in the ABR. The COD organic loading rate and the F/M ratio were approximately 0.20 ± 0.02 g COD/(l day) and 0.006 g COD/(g VSS day), respectively, in the ABR reactor. The hydraulic retention time was adjusted to 2 days by a peristaltic pump while the solid retention time was calculated as 98 days. During the anaerobic phase the dissolved oxygen was zero, the pH and the redox potential were around 7.8 and −360 mV, respectively.

2.4.2. Treatment of pharmaceutical wastewater in sequential ABR and CSTR reactor system

The Kemicetine concentrations varied between 32 and 125 mg/l in the real pharmaceutical wastewater depending on the process of the antibiotic production. The influent COD concentrations, the COD organic loading rate and the F/M ratio were approximately 3000 ± 450 mg/l and 0.23 ± 0.09 g COD/(l day) and 0.009 g COD/(g VSS day), respectively, throughout the anaerobic operation. The hydraulic retention time was kept constant at 1.2 days in the ABR [41] while the solid retention time was calculated as 45 days, respectively. The resistance of granular sludge to high Kemicetine concentrations could be attributed to the compact structure of acclimated anaerobic cells during granulation in ABR. This biomass could be regarded as different forms of cell immobilization. This facilitated the further strengthening of cell–cell interaction and resulted in the high density of adhering cells. As a result, the anaerobic bacteria formed granules through self-immobilization and the anaerobic sludge was converted to a relatively active granular sludge by enhancing agglomeration. This promoted granulation in the ABR. The granules exhibited mild resistance to the toxicity of Kemicetine, probably due to the granules layered microstructure and to a matrix to which the non-filamentous bacteria were attached. Furthermore, acclimatization of bacteria in the granules to Kemicetine and the tolerance of immobilized cells to high Kemicetine oncentrations in the ABR increased the resistance of granulated sludge to Kemicetine. Biogranules in the ABR enabled a high biomass retention and withstood high-strength wastewater and toxic loadings. None of the individual species in these microecosystems is capable of completely degrading the influent wastes. Enhanced granulation processes are desirable to reduce the space time requirements of bioreactors. It has been observed that anaerobic granulation can proceed well at a relatively short HRT combined with a up and down flow liquid velocity. This can lead to a decrease in non-granulation competent bacteria by promoting sludge granulation in the ABR.

No additional nitrogen and phosphorous were administered to the raw wastewater since the BOD/N/P ratios were around 100/3/1. No sludge wasting was applied in the ABR during continuous operation.

Sludge retention time in the aerobic CSTR was adjusted to 20 days. During the aerobic phase the dissolved oxygen was adjusted below 2 mg/l and the redox potential was around +100 mV.

2.5. Analytical procedure

2.5.1. Measurement of conventional parameters

pH was measured daily in the inlet and outlet of the reactors with a pH meter probe. Temperature was measured daily in the ABR reactor inlet and outlet with an electronic temperature meter. HCO_3 alkalinity was measured 3 times per week in the ABR reactor influent and effluent using the Anderson–Yang method [42]. Volatile fatty acid (VFA) was measured in the effluent of the ABR 3 times per week using the Anderson–Yang Method [42]. Total and methane gases were measured daily in the top of the ABR reactor using the liquid-displacement system [43,44]. Soluble COD in the influent and effluent of the ABR reactor and CSTR was measured twice per week in centrifuged samples [45]. TSS, MLSS, VSS and MLVSS were measured in the ABR and CSTR reactors by the membrane filtering method [45]. Dissolved oxygen was measured 4 times per week in the ABR reactor effluent and the CSTR reactor using a WTW-OX 330 oxygenmeter. Biological oxygen demand (BOD5) was measured 4 times per week in the ABR reactor effluent using WTW OXI pumps [45]. Methane percentage was measured daily using Digital Drager Pac Ex2 apparatus. Kemicetine and intermediate products were measured in a Hewlett Packard (HP) gas chromatography mass spectrum (GC–MS) with a diode array detector using a supelcosil C-18 column (Supelco, USA). The effluent consisted of a methanol/water mixture (60/40) and the detector wavelength was 284 nm [45].

Phenol, NH_4–N and Cl^{-1} were measured following standard methods [45].

2.5.2. Measurements of specific parameters

Soluble inert COD was measured twice per week in the ABR reactor outlet using the glucose comparison method [46]. This method involves running three batch reactors, two with the wastewater to be studied and the third with glucose. One of the wastewater reactors has the total COD, and the second has the total soluble COD, whereas the initial COD in the glucose reactor is adjusted to equal COD value. The experimental studies are performed until all the biodegradable COD is depleted, where the COD profiles reach a plateau and stay unchanged. The difference betwen glucose COD and wastewater COD gives the inert COD.

Readily biodegradable COD was measured 2 times per week in the ABR reactor effluent from the equivalent (dissolved O_2 change/$(1-Y)$) [46]. Y is the yield and it is equal to the ratio [produced biomass (VSS)/removed soluble COD] which was obtained from the experimental data. Y-Values were calculated as 1100/2668 = 0.41 g VSS/g COD, 800/2407 = 0.33 g VSS/g COD, and 600/2092 = 0.28 g VSS/g COD through Runs 1, 2 and 3, respectively. If the mean dissolved oxygen change was 1780 mg/l based on COD and the mean Y was 0.30 g VSS/g COD and the COD originating from the readily degradable organic compounds was calculated as 2540 mg/l in Run 1. Slowly biodegradable COD was measured 2 times per week in ABR reactor outlet which is equivalent to [soluble COD − (readily biodegradable COD + inert soluble COD)] [46]. If the soluble COD, readily degradable COD and the inert microbial products COD concentrations were measured as 2490, 2360 and 70 mg/l, the slowly degradable COD was calculated 120 mg/l in Run 1. The COD originating from the microbial products was calculated from the [(final soluble COD (remaining COD) (after BOD5 measurement) − (soluble inert COD)] [46].

Anaerobic toxicity assay (ATA) was performed to evaluate the toxicity of the increasing Kemicetine concentrations (from 1, 10, 25, 50 to 100 mg/l) to anaerobic microorganisms in order to obtain the Kemicetine dose to be used through continuous operation of a sequential ABR/CSTR reactor system. The serum bottles containing 15 ml of unacclimatized sludge, 20 ml sodium thioglycollate, 4000 mg/l $NaHCO_3$, 3000 mg/l glucose and increasing Kemicetine concentrations were incubated at 37 °C. 50% reduction in gas productions compared to the control samples in serum bottles was defined as IC_{50} value [47,48].

2.6. Toxicity measurements

2.6.1. LUMIStox (Microthox) toxicity assay

A specific strain of the marine bacterium *P. phosphoreum* was used in this test to determine the toxicity of p-NP and NB. Reductions in light intensity at 5th, 10th and 30th min were chosen to measure the toxicity [49]. The standard culture, *P. phosphoreum* (LCK480), was obtained from Dr. Lange (Germany). Microtox testing was performed according to the standard procedure recommended by the manufacturer [49]. The bioluminescense of the sample was measured in a luminometer (LUMIStox). Before the toxicity assay, the pH of the sample was adjusted to between 5.5 and 8.5 using 0.1N NaOH or HCl. Room temperature was maintained at between 15 and 24 °C. Samples were serially diluted with 2% NaCl (w/v). Sodium chloride (2%) was used as the control. Samples containing bacterial luminescence were measured for 5, 15 and 30 min incubation times in a luminometer. The decrease in bioluminescence indicated the toxic effect of the samples. Toxicity evaluation criteria for luminescent bacteria are explained by the percent inhibition effect (H). If the percent inhibitory effect (H) changes between 0% and 5%, the effect is non-toxic. When it is between 5% and 20%, the effect is possibly toxic, and when the inhibitor effect is between 20% and 90%, the effect is toxic [49].

2.6.2. D. magna toxicity test

Toxicity was tested using 24 h born *D. magna* as described in Standard Methods [45]. Test animals were obtained from the Faculty of Science, Ege University, Izmir, Turkey. After preparing the test solution, experiments were carried out using 5 or 10 *Daphnids* introduced into the test vessel. These vessels were controlled with 100 ml of effective volume at 7–8 pH, providing a minimum dissolved oxygen concentration of 6 mg/l at an ambient temperature of 20–25 °C.

Young *D. magna* were used in the test (in first start ≤24 h old). A 24 h exposure is generally accepted for a *Daphnia* acute toxicity test. Results were expressed as mortality percentage of the *Daphnids*. The immobile animals were determined as dead *Daphnids*.

2.6.3. Chlorella (algae) toxicity

Chlorella were obtained from the Faculty of Science, Ege University, Izmir, Turkey. 10 *Chlorella* were placed in 2 l of wastewater diluted at ratios varying between 1/2, 1/3, 1/4, 1/5 and 1/6. After 48 h of incubation period a lack of movement in the *Chlorella* was evaluated as acute toxicity.

2.7. Statistical analysis

Analysis of variance (ANOVA) between experimental data was performed to detect the F and p-values. In other words, the ANOVA test was used to test for differences between dependent and independent groups [50]. The comparison between the actual variation of the experimental date averages and standard deviation was expressed in terms of F ratio. F was equal to "found variation of the date averages/expected variation of the date averages". p reported the significance level. Regression analysis was applied to the experimental date in order to determine the regression coefficient R^2.

The Kruskal–Wallis one-way non-parametric significance tests were used to detect the differences between organism used in the acute toxicity tests. All the statistical analysis were conducted with STATGRAPHICS Centurion XV, software [51].

3. Results and discussion

3.1. Batch anaerobic toxicity test (ATA) results in the ABR

The ATA test was carried out at increasing Kemicetine concentrations.

In this study, inhibition is defined as a reduction in the activity, in terms of gas production, of a batch study relative to its activity before the addition of Kemicetine. The concentration of Kemicetine resulting in 50% inhibition of the rate of production (IC_{50}) of methane gas was found to be 95 mg/l. In other words, the concentration of Kemicetine resulting in 50% inhibition of the produced rate of methane gas (IC_{50}) was 95 mg/l (Fig. 1). Fernández et al. found that the anaerobic ammonium oxidation (Anammox) process was feasible for treating wastewaters containing chloramphenicol ranging from 20 to 1000 mg/l, tested in batch assays [52]. A strong inhibitory effect was observed while the IC_{50} value was found as 35 mg/l. Lu et al. found that the degradation rate of 60 mg/l chloramphenical was 29% and the IC_{50} value of the chloramphenicole (varying between 20 and 200 mg/l) was 43 mg/l when pure culture of *Pseudomonas aeruginosa* was used as seed [53]. These results showed that the anaerobic partially granulated culture used in our study is more resistant than that used in the aforementioned studies since their IC_{50} values are lower. The anaerobic partially granulated sludge used as seed in this study was not affected significantly by the increasing Kemicetine concentrations, compared to the literature data given above.

3.2. ABR reactor performance in the start-up period

The COD removal efficiencies were 20% and 88% after operation times of 7 and 21 days, respectively. The methane gas production and methane percentage were approximately 24 mg/l and 10% at the beginning of the start-up period. The methane gas production and methane percentage reached 1120 ml/day and 55%, respectively, after 10 days of the operation time. The COD removal efficiencies remained stable at 92% after an operation period of 30 days. The daily methane gas production and methane percentage remained stable at 2500 ml/day and 71%, respectively, after 30 days of the start-up period before feeding with raw pharmaceutical wastewater containing Kemicetine.

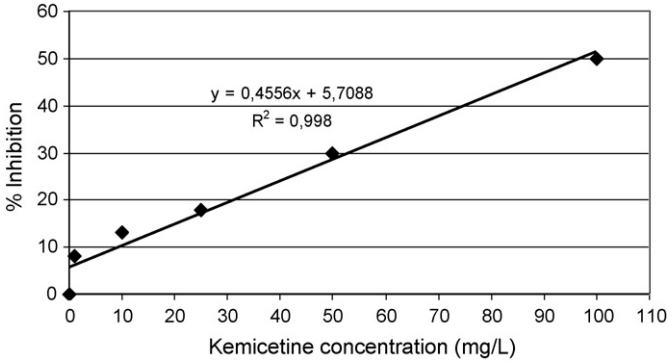

Fig. 1. ATA test results for Kemicetine (IC_{50} = 95 mg/l).

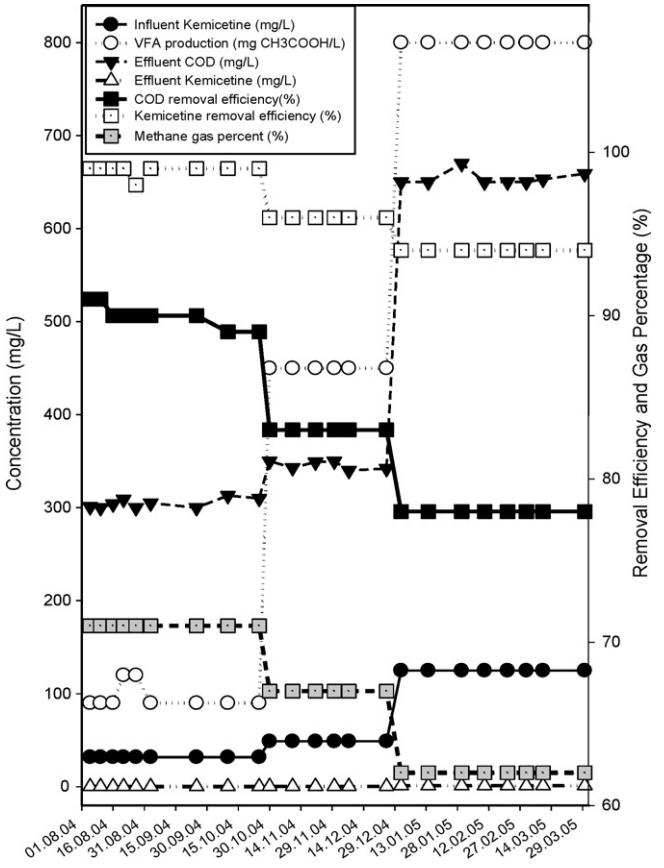

Fig. 2. The variations of COD, Kemicetine removal efficiencies, VFA concentrations and methane percentages at increasing Kemicetine concentrations in ABR.

3.3. Effect of increasing Kemicetine concentrations on COD, Kemicetine, VFA removals, and methane gas percentages in the ABR

The operation of the ABR was started with real pharmaceutical wastewater containing 32 mg/l Kemicetine (Run 1) (see Table 1). No significant changes in COD removal efficiencies were obtained at this Kemicetine concentration. Kemicetine removal efficiency was approximately 98–99% in Run 1(see Fig. 2). The volatile fatty acid (VFA) concentrations fluctuated throughout the start-up period (230 mg CH_3COOH/l, data not shown). After 30 days, the VFA concentration reached a maximum value of 80 mg CH_3COOH/l in Run 1. In this run, the VFA concentrations varied at between 90 and 120 mg CH_3COOH/l (see Fig. 2).

Pharmaceutical wastewater characterization is now regarded as an indispensable step yielding all the necessary information for a reliable modelling and design of anaerobic and aerobic processes, particularly for the treatment of toxic and refractory organics. The amount of organic carbon is only meaningful when it is expressed in terms of various fractions with different mechanisms and rates of biodegradation. In this respect, COD fractionation has been introduced as a very useful tool for the evaluation of anaerobic ABR and aerobic CSTR reactor processes treating pharmaceutical industry wastewater. COD fractionation involves the identification of the CODs originating from the inert compounds together with readily biodegradable and slowly biodegradable organics. The COD originating from the readily biodegradable organic compounds is hypothesized to consist of simple soluble molecules that can be readily absorbed by the organisms, whereas the slowly biodegradable substrates are assumed to be made up of particu-

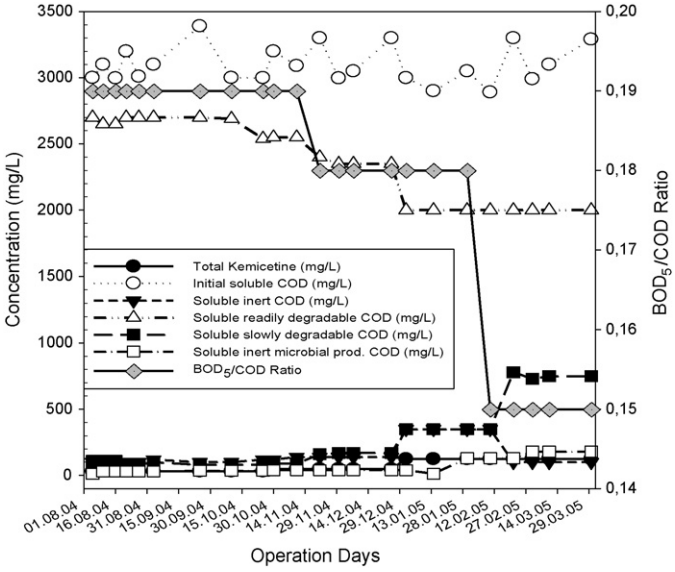

Fig. 3. COD subcategories and BOD5/COD ratios in anaerobic ABR influent.

late/colloidal/complex organic molecules that require enzymatic breakdown prior to absorption and utilization. The COD originating from the inert fractions of the wastewaters is of importance in meeting the discharge limitations as it by-passes the biological treatment system without being affected by the biochemical reactions and becomes the major constituent of the effluent. The inert fraction may be further subdivided into soluble inert COD (this fraction in the influent by-passes the system without affecting the biochemical reactions in the reactor) and particulate inert COD (this fraction is initially present in the wastewater or metabolically produced during the aerobic treatment and leaves the process with excess sludge). In the anaerobic hydrolysis the COD originating from the slowly degradable compounds is transformed into COD originating from the readily degradable organics and a small fraction of COD originating from the inert compounds. The soluble microbial products generated by the hydrolysis of slowly degradable organics to readily degradable organics and by the decay of biomass through endogenous phase are directly converted into stored material in bacterial cells. These stored compounds are subsequently used as a carbon and energy source for growth purposes. Bacteria in the anaerobic/aerobic processes might be able to utilize directly the Kemicetine with COD and the aforementioned stored components of COD. The influent total COD of the wastewater consisted of the CODs originating from the readily degradable organics (2540–2700 mg/l), and from the inert and slowly degradable substances (80 and 120 mg/l, respectively), in Run 1 (see Figs. 2 and 3). Fig. 4a shows the calculation of 80 mg/l inert COD concentration measured in this run under constant glucose-COD and wastewater COD after one month of bach tests. The high removals in COD could be attributed to the high concentration of COD originating from the readily degradable organics in the influent of the ABR in the period between 1st August 2004 and 25th November 2004 (see Figs. 2 and 3).

When the Kemicetine concentration was 49 mg/l in the raw pharmaceutical wastewater (Run 2), the COD removal efficiency was reduced and remained at approximately 83% (see Fig. 1). The Kemicetine yield was 96% in this run. The VFA concentration was approximately 450 mg CH_3COOH/l (see Fig. 2). The methane percentage decreased to 67% in this run. At the beginning of this run, the concentration of readily degradable COD decreased from 2700 to 2350 mg/l. The COD concentrations originating from the inert and slowly degradable organics did not vary significantly in the

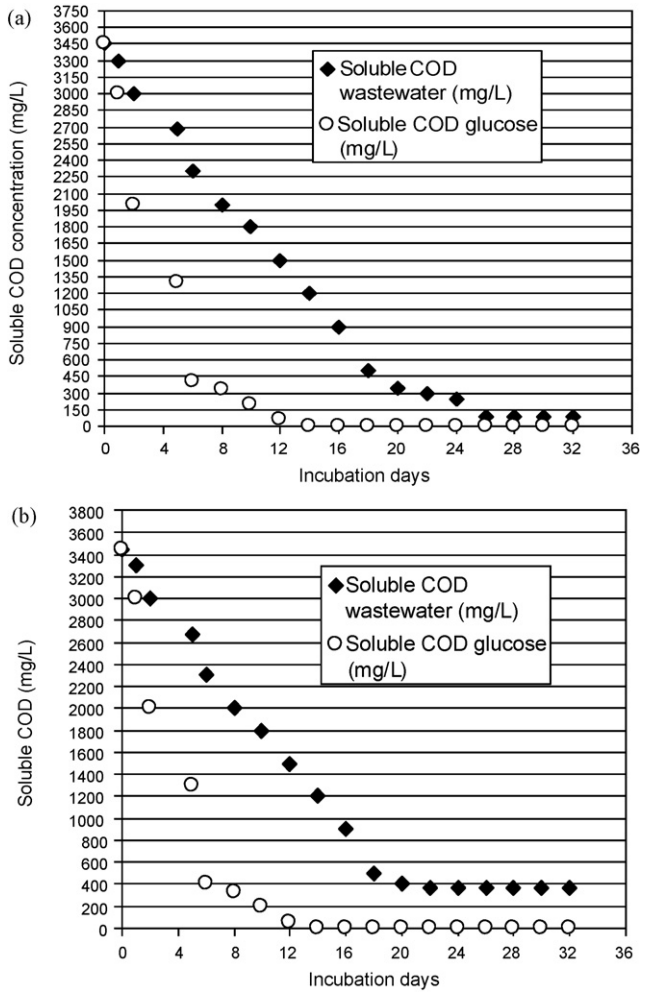

Fig. 4. Inert COD concentration in Run 1 at a Kemicetine concentration of 32 mg/l (80 mg/l, (a)) inert COD concentration in Run 3 at a Kemicetine concentration of 125 mg/l (368 mg/l, (b)).

influent samples of ABR during this period (see Fig. 3). During the period between 30th October 2004 and 25th December 2004, although the COD originating from the readily degradable organics was degraded highly efficiently (98%), the COD concentrations originating from the slowly degradable organics and inert components increased in the effluent samples of the ABR. Therefore the COD removals decreased in Run 2 compared to Run 1 (see Figs. 3 and 5).

When the ABR reactor was operated with a Kemicetine concentration of 125 mg/l (Run 3), the COD removal efficiencies were approximately 78%. The VFA concentrations were measured as 800 mg CH_3COOH/l. The methane percentage of the total biogas decreased to 62%. The Kemicetine yield was reduced to 94% (see Fig. 2). At the beginning of this run, the COD concentrations originating from the slowly degradable organics increased from 170 to 780 mg/l, while the concentrations of COD originating from the readily degradable organics decreased to 1700 mg/l in the influent samples. The COD originating from the inert compounds also increased from 100 to 180 mg/l in the influent samples of the ABR reactor (see Figs. 3 and 5). The other COD components in the influent were composed of the COD originating from the 300–350 mg/l of slowly degradable organics and from the 310–368 mg/l of inert microbial products during the period between 1st January 2005 and 30th January 2005 (see Fig. 3). Fig. 4b illustrates the calculation of the 368 mg/l inert COD concentration measured in this run after reaching a stable plateau for glucose-COD and wastewater COD.

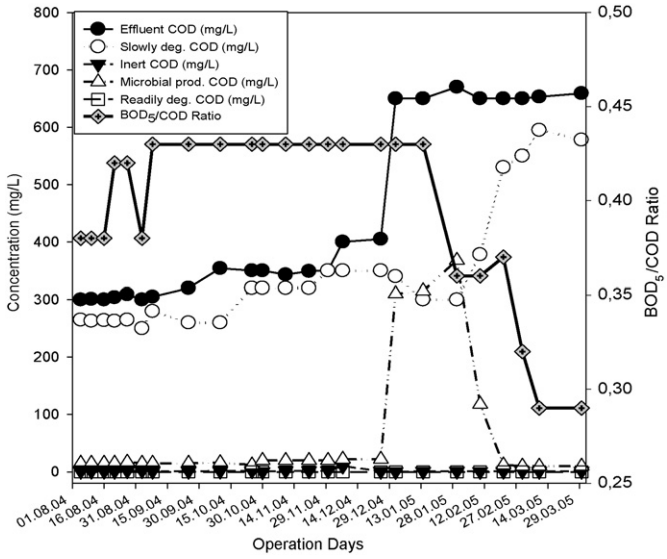

Fig. 5. The COD categories and BOD5/COD ratios in anaerobic ABR effluent.

The Kemicetine concentrations in the effluent of the ABR were measured as 0.32, 0.49 and 1.25 mg/l, respectively, at the end of three runs. Kemicetine was degraded to two main metabolites, namely amino-1 (p-nitrophenil)-1,3 propanediol and p-amino phenyl. Since a large proportion of Kemicetine was transformed into the aforementioned metabolites, high Kemicetine removals were obtained in the ABR reactor. The low COD removals in the ABR reactor could be attributed to Kemicetine metabolites since they were not removed in the ABR reactor. Therefore, they were measured as COD in the effluent of the anaerobic ABR reactor. This increased the COD concentrations in the effluent of the ABR reactor. Although these metabolites are readily degradable under aerobic conditions they were not removed in the ABR reactor since some heterothophic and methanogenic bacteria in the ABR were not able to use the amino-1 (p-nitrophenil)-1,3 propanediol and p-amino phenyl as substrate under anaerobic conditions.

In this study it was found that the anaerobic ABR reactor was primarily used for high Kemicetine removals and secondly contibuted to COD removals. Low COD yields obtained at high Kemicetine concentration ABR was dependent on COD components in the influent wastewaters. The reason for the low COD removals was the CODs originating from the slowly degradable organics and inert compounds in the influent wastewater. Since high Kemicetine concentrations in the ABR reactor converted into corresponding metabolites, Kemicetine was removed with high yields varying between 94% and 96% in Runs 2 and 3, respectively. Therefore, the low COD removals did not depend on high concentrations of Kemicetine. It has been mentioned that some COD components like inert and slowly degradable organics in pharmaceutical wastewaters significantly affect the reactor performance [54–56]. Based on this information, it is important to determine the relationships between COD components, reactor performances and toxicity removals.

The results of this study showed that the ABR reactor exhibited high COD and Kemicetine removal efficiencies in the treatment of pharmaceutical wastewater when the influent wastewater contained high concentrations of CODs originating from the readily degradable organics and low concentrations of CODs originating from the slowly degradable organics and inert compounds. At high Kemicetine concentrations the COD and Kemicetine removals could be low if the pharmaceutical wastewater contained high concentrations of CODs originating from the slowly degradable and inert compounds in the influent of ABR. It is important to note that when the Kemicetine concentrations were high, Kemicetine did not inhibit the anaerobic treatment process in the ABR reactor since it converted into corresponding metabolites. In this study it was found that at low Kemicetine concentrations and also if the influent wastewater contained high concentrations of COD originating from the slowly degradable and from the inert compounds the anaerobic reactor performance decreased significantly. The low COD removals were not dependent on high Kemicetine concentrations in the ABR reactor while the components of COD were primarily related to the reactor performance.

In this study the COD, Kemicetine removal efficiencies, methane percentages of biogas and the specific methanogenic activity (SMA) values found in the anaerobic ABR reactor were significantly higher than those in studies performed by Sreekanth et al. [57] in an anaerobic sludge blanket reactor treating pharmaceutical wastewater containing drug the Paracetamol (65–75% COD removal with biogas production containing 60–70% of methane and a SMA of 320 ml CH_4/(g VSS day), by Zhou et al. [58] in a hybrid UASB reactor treating chemical-based antibiotic wastewater (72% COD removal efficiency and a SMA value of 200 ml CH_4/(g TVS day) (in this study, 890 ml CH_4/(g VSS day)), data not shown), by Chelliapan et al. [59] in an upflow anaerobic stage reactor (UASR) treating antibiotics (%85, %75 and %75 tylosin, avilamysin and COD removals, respectively) and by Sponza and Demirden [60] in an UASB reactor treating sulfamerazine (89% sulfamerazine and 82% COD removals).

3.4. The BOD5/COD ratios in anaerobic reactor influent and effluents

The biodegradability of an industrial wastewater is dependent upon BOD5/COD ratio. It has generally been accepted that when a biodegradability ratio is greater than 0.3, this represents a readily biodegradable effluent [31]. An increase in the BOD5/COD ratio indicates an improvement in the biodegradability of the pharmaceutical wastewater containing Kemicetine due to formation of inter-metabolite-products more biologically degradable, as reported by Carballa et al. [61] and Çokgör et al. [54]. In this study, the BOD5/COD ratio in the influent wastewater of the ABR varied between 0.15 and 0.19 (see Fig. 3). Fig. 5 shows the BOD5/COD ratios obtained in the effluent of the ABR. The BOD5/COD ratios in the effluent of the ABR increased from 0.15 to 0.31 and from 0.19 to 0.43, respectively, indicating the biodegradability of the wastewater after anaerobic treatment. The anaerobic transformation of Kemicetine into more biodegradeble inter-metabolites in the ABR reactor increased the biodegradability ratio of the pharmaceutical wastewater.

3.5. Intermediate products in ABR and in CSTR reactors

The GC–MS analysis showed that 12.24 mg/l 2-amino-1 (p-nitrophenil)-1,3 propanediol and 14.5 mg/l p-amino phenyl were detected when the ABR reactor was operated with a Kemicetine concentration of 49 mg/l in the period between 1st January 2005 and 14th January 2005 (data not shown). These metabolites were also determined by the National Toxicology Program board in the anaerobic treatment of Kemicetine [62]. Furthermore, 3.2 mg/l p-amino phenol, 2.6 mg/l phenol, 1.9 mg/(l Cl^{-1}) and 1.32 mg/l ammonia were detected in the effluent of the ABR. The Kemicetine was degraded with a removal efficiency of 96% and an effluent Kemicetine concentration of 0.32 mg/l in the effluent of the ABR (see Figs. 2 and 5). The 2-amino-1 (p-nitrophenil)-1,3 propanediol and l-p-amino phenyl concentrations were measured as 29 and 35 mg/l at a Kemicetine concentration of 125 mg/l. The p-amino phenol, phenol, Cl^{-1} and ammonia concentrations were 5.5, 3.2, 2.1 and 2.4 mg/l, respectively, at this Kemicetine concentration. The concentrations of propanediol and p-amino phenyl were

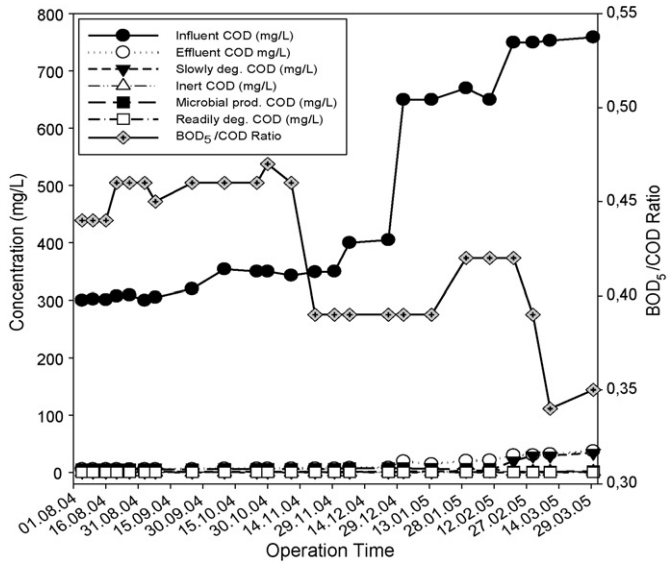

Fig. 6. The COD subcategories and BOD5/COD ratios in Aerobic CSTR effluent.

measured as zero in the effluent of the aerobic reactor in the period between 1st January 2005 and 14th January 2005. No p-amino phenol, phenol, Cl^{-1} or ammonia was detected in the effluent of the aerobic reactor. Kemicetine was completely degraded with an effluent Kemicetine concentration of zero in the effluent of the aerobic reactor. It can be concluded that the aerobic CSTR reactor contibutes to the removal of all the inter-medite organics produced through anaerobic treatment in the ABR, the Kemicetine and the COD remaining from the anerobic ABR reactor.

The total COD concentrations of the intermediate organics produced from Kemicetine through anaerobic treatment in the ABR were calculated at betwen 29 and 110 mg/l. They were considered mainly as readily degradable-COD [41] since they were removed completely in the aerobic reactor. The COD concentration of 125 mg/l Kemicetine was measured as 110 mg/l. 89% of this COD (97 mg/l) originated from the intermediate products which were measured as readily degradable organics. The remaining COD of the intermediate products consisted of the inert microbial products (1.9 mg/l) and of the slowly degradable organics (12 mg/l) in the period between 21st February 2005 and 30th March 2005 [41]. This explained the fact that the low COD removals in the anaerobic ABR reactor were not caused by the intermediate products of Kemicetine.

3.6. Relationships between COD subcategories and BOD5/COD ratio in aerobic reactor effluent

The slowly degradable COD concentrations were measured at between 20 and 29 mg/l between 21st February 2005 and 30th March 2005 in the effluent of the aerobic reactor (see Fig. 6). The inert COD originating from the microbial extracellular products in the pharmaceutical wastewater under anaerobic conditions for the aforementioned days is also shown in Fig. 5. This component of COD decreased significantly (removal efficiency = 98%) in the effluent of the aerobic reactor. The COD concentration measured in the effluent of the aerobic reactor (25–32 mg/l) mainly originated from the 24–29 mg/l of slowly degradable COD in the effluent of the aerobic reactor in the period between 1st January 2005 and 30th January. The low COD removals (70–75%) between the aforementioned days could be attributed to the COD originating from the slowly degradable organics in aerobic reactor (see Fig. 6). The COD originating from the readily degradable organic compounds was removed completely in the anaerobic reactor.

The BOD5/COD ratio increased from 0.31 and 0.43 to 0.37–0.49 in the effluent of the aerobic reactor. This ratio indicates the highly biodegradable structure of the pharmaceutical wastewater in the aerobic CSTR.

3.7. Acute toxicity test results in the effluent of anaerobic and aerobic reactors

Acute toxicity tests were performed with three different organisms in order to determine three separate trophic levels (water flea *D. magna* in the *D. magna* toxicity test, *Photobacterium photospherium* in the Microthox toxicity test, and *Chlorella* (algae) in the toxicity tests. The results showed that acute toxicity was not present in the period between 5th August 2004 and 25th November 2004 in the effluent of the ABR (see Table 2).

The acute toxicity test results showed that potential acute toxicity was detected in the raw pharmaceutical wastewater samples containing Kemicetine concentrations from 32 to 125 mg/l in all trophic organisms used (data not shown) in the period between 1st January 2005 and 21st February 2005. Slightly acute toxicity was observed in the effluent samples of the ABR. Since the Kemicetine transformed into readily degradable intermediate products and degraded with high removal efficiency, the toxicity on the aforementioned days could be attributed to the COD components originating from the inert and from the slowly degradable compounds in the effluent of the ABR (Table 2). It is assumed that the COD originating from the inert microbial products did not cause toxicity, since the concentrations was low (see Fig. 5). In the period between 21st January 2005 and 30th March 2005, moderately acute toxicity was observed. This toxicity could only be attributed to the high concentration of COD originating from the slowly degradable organics in the effluent of the ABR. No acute toxicity was observed for sampling days between 05th July 2004 and 21st February 2005 in the effluent samples of the aerobic reactor (see Table 3). Slightly acute toxicity was observed between days 30th February 2005 and 10th March 2005. This toxicity could be attributed to the 29 mg/l COD originating from the slowly degradable organics in the effluent of the aerobic reactor. Since the COD concentration originating from the inert microbial products and other inert compounds was low (1–2 mg/l), it did not cause toxicity (see Table 3). The moderate toxicity was also dependent on the 34 mg/l COD originating from the slowly degradable organics on sampling day 30th March 2005.

The slight toxicity was dependent upon a high concentration of COD originating from the slowly degradable organics and and inert microbial products in the effluent of the aerobic reactor in the period between 1st January 2005 and 10th February 2005 (see Table 3). The moderate toxicity originated only from the slowly degradable COD in the next period. The acute toxicity and low COD removal efficiencies in Run 3 could be attributed to the high cencentrations of COD originating from the slowly degradable organics, in contrast to the studies performed by Eremektar et al. [63]. In their studies, the low COD removals and toxicity were explained by high inert COD concentrations in pharmaceutical wastewaters.

In our study, toxicity was mainly dependent on high COD concentrations originating from the slowly degradable organics and, more rarely, the low COD concentrations originating from the inert components on some days.

The statistical significance between the acute toxicities of three organism was determined non-parametrically using the Kruskal–Wallis test [51]. This test statistics revealed that there were significant differences between acute toxicities of *Chlorella*, water flea and bacteria ($p = 0.0001$). The *Chlorella* had higher EC_{50} values than *D. magna* and *Photobacterium phospherium* bacteria in the influent samples ($p = 0.001$) [51]. This showed that *D. magna* and

Table 2
Acute toxicity test results in the anaerobic ABR reactor effluent.

Days	EC_{50} value (%, w/v)			General results
	Daphnia magna	Microthox	Algae (*Chlorella*)	
05.08.2004	99	100	100	No acute toxicity
10.08.2004	100	100	100	No acute toxicity
6.08.2004	100	100	100	No acute toxicity
21.08.2004	100	100	100	No acute toxicity
27.08.2004	100	100	100	No acute toxicity
03.09.2004	100	100	100	No acute toxicity
08.09.2004	100	100	100	No acute toxicity
25.09.2004	100	100	100	No acute toxicity
10.10.2004	100	100	100	No acute toxicity
25.10.2004	100	100	100	No acute toxicity
30.10.2004	100	100	100	No acute toxicity
10.11.2004	100	100	100	No acute toxicity
21.11.2004	100	100	100	No acute toxicity
30.11.2004	100	100	100	No acute toxicity
07.12.2004	99	99	100	No acute toxicity
25.12.2004	98	98	98	No acute toxicity
01.01.2005	99	98	100	Slightly acute toxicity
14.01.2005	96	97	100	Slightly acute toxicity
30.01.2005	93	97	100	Slightly acute toxicity
10.02.2005	98	99	100	Slightly acute toxicity
21.02.2005	14	28	100	Moderate acute toxicity

Table 3
Acute toxicity test results in the aerobic CSTR reactor effluent.

Days	EC_{50} value (%, w/v)			General results
	Daphnia magna	Microthox	Algae (*Chlorella*)	
05.08.2004	100	100	100	No acute toxicity
10.08.2004	100	100	100	No acute toxicity
16.08.2004	100	100	100	No acute toxicity
21.08.2004	100	100	100	No acute toxicity
27.08.2004	100	100	100	No acute toxicity
03.09.2004	100	100	100	No acute toxicity
08.09.2004	100	100	100	No acute toxicity
25.09.2004	100	100	100	No acute toxicity
10.10.2004	100	100	100	No acute toxicity
25.10.2004	100	100	100	No acute toxicity
30.10.2004	100	100	100	No acute toxicity
10.11.2004	100	100	100	No acute toxicity
21.11.2004	100	100	100	No acute toxicity
30.11.2004	100	100	100	No acute toxicity
07.12.2004	96	96	95	No acute toxicity
25.12.2004	98	98	98	No acute toxicity
01.01.2005	100	100	100	No acute toxicity
14.01.2005	100	100	100	No acute toxicity
30.01.2005	100	100	100	No acute toxicity
10.02.2005	100	100	100	No acute toxicity
21.02.2005	100	100	100	No acute toxicity
30.02.2005	78	49	99	Slightly acute toxicity
10.03.2005	34	28	99	Slightly acute toxicity
30.03.2005	9	9	97	Slightly acute toxicity

Microthox acute toxicity tests are the most sensitive, and *Chlorella* is the most resistant organism to the pharmaceutical wastewater containing Kemicetine.

The *D. magna* and *Photobacterium phospherium* bacteria used in Microthox tests had similar toxicity responses in both anaerobic and aerobic reactor effluents for pharmaceutical wastewaters. The Kruskal–Wallis test for non-normally distributed data indicates a non-significant difference at $p = 0.001$ (see Tables 4 and 5) [51]. This could be explained by the sensitivity of both organisms used in acute toxicity tests to the effluents of ABR and CSTR reactors. The EC_{50} values measured in *Chlorella* were higher than those for *D. magna* and *Photobacterium phospherium* bacteria and these differences were significant (see Tables 4 and 5). The Kruskal–Wallis test for non-normally distributed data indicates a significant difference at $p = 0.001$ [51]. This showed that *Chlorella* was resistant to the effluents of both reactors.

In the present study, *P. phosphoreum* bacteria and *D. magna* have similar toxicity responses to the pharmaceutical industry wastewater, while algae have lower sensitivity scores. In other words, the acute toxicity test results indicated that the EC_{50} values measured for algae differed from the *D. magna* and Microthox tests. The algae exhibited lower mortalities with higher EC_{50} values, resulting in a more resistant organism than that of the *D. magna* and Microthox tests.

3.8. Cost estimation in ABR and CSTR reactors

An estimation of costs has been made regarding the capital and operating costs for the treatment process used for the treatment of pharmaceutical industrial wastewater containing Kemicetine. Anaerobic systems such as the ABR have the ability to recover and use biogas. For anaerobic treatment systems the electricity

Table 4
Comparison of organism sensitivities used in acute toxicity tests using Kruskal–Wallis test statistic (KW-T) in the anaerobic reactor effluent.

	Daphnia magna	Microthox	Algae (*Chlorella*)
Daphnia magna		S KW-T = 12.9, $p = 0.001$	N.S KW-T = 0.05, $p = 0.001$
Microthox			N.S KW-T = 0.04, $p = 0.001$

KW-T: Kruskal–Wallis test statistic; S: sensitive; N.S: not sensitive.

Table 5
Comparison of organism sensitivities used in acute toxicity tests using Kruskal–Wallis test statistic (KW-T) in the aerobic reactor effluent.

	Daphnia magna	Microthox	Algae (*Chlorella*)
Daphnia magna		S KW-T = 15.99, $p = 0.001$	N.S KW-T = 0.54, $p = 0.001$
Microthox			N.S KW-T = 0.98, $p = 0.001$

KW-T: Kruskal–Wallis test statistic; S: sensitive; N.S: not sensitive.

consumption is lower since only pumping costs are incurred. The operation and maintenance costs in the anaerobic ABR reactor are compensated for by the methane gas production and this amount is proportional to the mass of organic matter removed. The CH_4 produced from the ABR treating 125 mg/l Kemicetine is equal to 0.025 m^3/day at a HRT of 12.8 days. The heat and electricity generation through methane utilization was 12.44 kW h/m^3. The energy yield produced from this methane was found to be 0.311 kW h. The quantity of the electric energy consumption in the dosage pums in ABR and in the air pump in CSTR reactor was 0.134 kW h. One third of the generated energy can be used to work the pumps instead of electricity. Furthermore, the remaining energy can be used to heat the ABR reactor and the studied research laboratories, and in the working of the pumps and some mechanical equipments.

For continuous anaerobic treatment of pharmacueutcal wastewater containing Kemicetine the overall costs were represented by the sum of the capital costs, the operating and the maintenance costs. $NaHCO_3$, sodium thioglycollate and treace minerals are continuously supplied for the growth of the biomass and the target maximum treatment efficiencies were 92% and 98% for COD and Kemicetine, respectively, in the ABR reactor. It is important to note that these costs strongly depend on the nature of the wastewater and on the concentrations of the pollutants, the flow rate of the effluent and the configuration of the reactor.

The total capital costs including the steinless-steel ABR reactor (8 USD) and the pumping (4.5 USD) was 12.5 USD. The pay-back period of the capital investment is estimated at around 1–2 years. The total operational cost, which consist of the chemical costs [$NaHCO_3$ alkalinity which is necessary to methanogens for a pH value around 7.7 (1.1 USD), sodium thioglicollate to maintain the anaerobic conditions (0.2 USD), trace minerals for methanogens (0.6 USD)] and electricity costs for heating the ABR to 37 °C (1.2 USD) was 3.1 USD. In conclusion, the total estimated operational cost was 3.1 USD for treating 30 m^3/day pharmaceutical industrial wastewaters containing 125 mg/l Kemicetine with removal efficiencies higher than 95%. This cost was very competitive when considering the operational cost of treated vinasse wastewater (a residue of ethanol fermentation) (1000 USD for 500 m^3/day)) [64]. The operational cost was very similar to that of Sreekanth et al. [57] and Sreekanth et al. [65] treating synthetic wastewater containing phenolic compounds and drug anatibiotic wastwater in the hybrid upflow anaerobic sludge blanket reactors (2.6 USD for a flow rate of 50 m^3/day).

4. Discussion

In this study, an increase in the biodegradability (BOD5/COD ratio) of pharmaceutical wastewater containing Kemicetine had already been observed. The same results were observed in a pharmaceutical wastewater containing penicillin formulation antibiotics [66]. The BOD5/COD ratio was improved from 0.15 and 0.19 to 0.37 and 0.49 via the anaerobic ABR/aerobic CSTR sequential reactor systems, respectively.

In this study, it was found that the COD originating from the readily degradable organics did not decrease the performance of the anaerobic ABR reactor since all the readily soluble organics were removed completely in the anaerobic stage. However, the CODs originating from the slowly degradable compounds and inert microbial products, in the pharmaceutical wastewater caused decreases in COD removals. In this study it was found that, although rarely, the COD originating from the inert componds did decrease the reactor performances. Similar results were found by Henze et al. and Ganesh et al. [67–69]. They reported that the soluble slowly degradable COD fraction is a rate limiting component for heterotrophophic growth in aerobic and anaerobic processes for pharmaceutical wastewater containing antibiotics. On the contrary, it was reported that only the residual inert COD (soluble inert COD + inert microbial products COD) are the key issue in the lowering of reactor performance for pharmaceutical wastewater [63].

It is important to note that in sequential systems, what is not biodegradable in one phase may be degradable in other phase. In this study, the Kemicetine was efficiently removed by transformation into its inter-metabolite products in the anaerobic ABR reactor. A large proportion of the COD was removed in this reactor. The anaerobic inter-metabolites of the Kemicetine were only degraded in the aerobic CSTR reactor. The COD and Kemicetine remaining from the anaerobic reactor were treated in the aerobic reactor.

In the present study, *P. phosphoreum* bacteria and *D. magna* have a similar toxicity response to pharmaceutical industry wastewater, while algae have higher sensitivity scores. In other words, the acute toxicity test results indicated that *D. magna* and Microthox tests are the most sensitive, and that *Chlorella* is the most resistant organism for the pharmaceutical wastewater containing Kemicetine. The EC_{50} values measured for algae differed from the *D. magna* and Microthox tests. The algae exhibited lower mortalities with higher EC50 values, resulting in lower sensitivity scores than the *D. magna* and Microthox tests.

Algae, water flea and bacteria acute toxicity test results demonstrated that the sequential ABR/aerobic reactor system eliminated the inhibitory effect of the pharmaceutical wastewater containing Kemicetine towards anaerobic and aerobic effluents.

The ANOVA test statistics showed that the correlation between y dependent (slightly and moderate acute toxicities) and *x* independent (CODs originating from the slowly degradable organics, from the inert microbial compounds and Kemicetine removals) variables is high, the relationships are strong and significant ($R^2 = 0.97$, $F = 89.23$, $p = 0.001$) [50] in the anerobic ABR reactor. Since the COD originating from the readily degradable organics were completely degraded in the anaerobic ABR reactor, this was not taken into consideration within the framework of this part of the statistical analysis.

On the other hand, the test statistics showed that the correlation between *y* dependent (slightly and moderate acute toxicities) and *x* independent (COD originating from the inert compounds, from the microbial products and from the slowly degradable organics) variables is low, the relationships are weak and not significant ($R^2 = 0.67$, $F = 7.11$, $p = 0.001$) [50] in the anerobic reactor.

The test statistics showed that the correlation between *y* dependent (slightly acute toxicities) and *x* independent (CODs originating from the inert compounds, from the inert microbial products and from the COD removals remaining from the anaerobic reactor) variables is low, the relationships are weak and not significant ($R^2 = 0.59$, $F = 5.99$, $p = 0.001$) [50] in the aerobic reactor.

It can be concluded that the acute toxicity depends strongly on the CODs originating from the slowly degradable organics and from the inert microbial products in the anaerobic reactor. Since the readily degradable organics are consumed by the microorganisms easily they do not affect the toxicity.

5. Conclusions

Although the IC_{50} value of Kemicetine was 95 mg/l, 125 mg/l Kemicetine was removed with an efficiency of 78%. The Kemicetine was mainly removed by transformation into corresponding intermediate products in a high rate anaerobic ABR reactor while a significant part of the COD originating from the readily degradable organics was also removed in this reactor. High methane percentages and low VFA concentrations were obtained in the ABR. The contribution of the aerobic reactor to the total removals of the sequential reactor system was the removal of COD and Kemicetine untreated in the anaerobic ABR reactor and the removal of the intermediate products produced from the anaerobic degradation of the Kemicetine.

Low removal efficiencies in total COD were firstly dependent on the CODs originating from the slowly degradable organics and secondly on the COD originating from the inert compounds in the ABR and CSTR reactors, since the transformed inter-metabolites of Kemicetine were mainly readily degradabile and they did not limit the anaerobic reactor performance.

D. magna and *P. phosphoreum* bacteria used in the Microthox test had similar toxicity responses (i.e. were sensitive) in anaerobic and aerobic reactor effluents, while *Chlorella* was resistant for pharmaceutical wastewaters containing Kemicetine. The ANOVA test statistics showed that the acute toxicities depended strongly on the CODs originating from the slowly degradable organics and from the inert microbial products in both reactors.

The sequential high rate anaerobic ABR/aerobic CSTR reactor is a promising process for treating pharmaceutical wastewaters containing antibiotics, and for removal of toxicity without a pretreatment stage.

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