Removals of Gentamicin and Benzo[a]Pyrene in an Anaerobic Multichamber Bed Reactor

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Abstract: The petrochemical industry wastewaters were not treated effectively due to high concentrations of Polycyclic aromatic hydrocarbon (PAH) benzo [a] pyren (BaP) and an aminoglucoside antibiotic gentamicin (GNT) in an aerobic treatment plant in Turkey. The high GNT concentration in this industry wastewater mainly originated from the toilets of the working people and from the medical care facilities since an intestinal epidemic occurrred for a period of approximately 1.5 years. The High BaP concentrations release into the wastewater originated from the high BaP production in this industry. In order to improve the biodegradability of petrochemical industry wastewaters containing excess BaP and GNT, different mixtures of BaP and GNT were biodegraded in a high rate anaerobic multichamber bed (AMCBR) reactor. The maximum anaerobic yields for 10 mg/L BaP and 1 mg/ L GNT alone were 45% and 12%, respectively. The addition of primary susbstrate increased the 200 mg/L BaP and 50 mg/ L GNT removals to 97% and 89%, respectively. BaP was biodegraded at short operation times compared to GNT. At low BaP (10-200 mg/ L) and GNT (10-35 mg/ L) concentrations, a non-competitive inhibition does not affect the binding of the substrate and K_S were not affected. At high BaP (500-900 mg/ L) and GNT (75-100 mg/ L) concentrations, the BaP and GNT were biodegraded according to competitive inhibition with increased K_S. BaP and GNT were biodegraded according to Haldane equations at high concentrations where they were used as the sole substrate.

Keywords: AMCBR, Benzo (a) pyrene, Inhibiton, Gentamicin, Petrochemical, Substrate.

1. INTRODUCTION

Even though PAHs are considered among the most difficult persistent organics to be treated because of their highly stable physical-chemical characteristics, high removal efficiencies were obtained with anaerobic treatment of low molecular PAHs namely acenaphthene, fluorene, phenanthrene, anthracene, carbazole [1, 2]. BaP is a typical carcinogenic 5-ring PAH [3]. Because of its low water solubility and high partition coefficient, this compound is hydrophobic and can not be successfully biodegraded in biological treatment plants [4]. The BaP yields were high in anaerobic biological treatment conditions (E=73%) as compared to the aerobic conditions (E= 54%) [5]. One of the aminoglycoside group antibiotics is GNT and it is effective against a wide variety of bacteria (pneumonia, typhus, and other bacteria-caused illnesses) and enteroviruses, and is believed to prevent the production of proteins in the invading bacterial cells [6,7]. There is little other information available on the occurrence and fate of aminoglycosides in wastewater and through treatment processes. However, due to their high sorption properties, it has been suggested that aminoglycoside antibiotics in wastewater would be adsorbed onto solid particles and colloidal organic matters [7]. GNT removals were around 3% at initial GNT concentration of 25 mg/ L under anaerobic conditions according to a 28-day long closed-bottle test [8]. 37% and 42% GNT yields were obtained in wastewater and in water containing 5% dextrose, respectively, after heat treatment. The metabolites of GNT were sisomicin and gentamine C1 [9]. According to the solubilities and the low Henry's law constants of PAHs, lower biodegradation yields would be expected for BaP compared to GNT [5, 10]. The GNT antibiotic becomes un-dissolved or partly dissolved in PAH mixtures with high benzene rings such as indeno[1,2,3-cd]pyrene, and BaP [11]. Antibiotics at high concentrations inhibit the microbial activities in wastewater treatment plants [12, 13]. Increasing solid retention times (SRTs) were reported to enhance the removal of several pharmaceutical compounds during biological processes [14]. The anaerobic treatabilities of hydrophobic PAHs and GNT antibiotic were high in the presence of primary substrates [15].

In the Aegean region of Izmir, some petroleum processing industries located in Aliağa district release their wastewater containing PAHs and antibiotics to the aquatic ecosystem, even though the wastewater was treated by aerobic conventional activated sludge system. The raw wastewaters of one of these industries contained 4-400 mg/ L BaP, 5-65 mg/ L GNT,

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0.007 µg/ L oxytetracycline and 0.009 µg/ L streptomycin. The high GNT concentration in this industry wastewater originated from an enterovirus epidemic, in which this antibiotic was used for the treatment of diarrhea, during a period of approximately 1.5 years.

The influent COD and biochemical oxygen demand (BOD_5) of this industrial wastewater were 1200-1300 mg/L and 345-367 mg/L, respectively, while the removal efficiencies of these parameters were low (47% for COD and 32% for BOD_5) in the effluent of the conventional aerobic activated sludge treatment plant. This showed that the wastewater containing PAHs and GNT antibiotic could not be removed effectively with this treatment. In the previous studies, the BaP yields in this industrial wastewater were increased by using 15 mg/ L rhamnolipid, surfactine and emulsan biosurfactants using an aerobic continuously stirred tank (CSTR) reactor [5]. However, this increases the cost of the treatment. The insufficient aerobic yields for BaP can be attributed to the high GNT antibiotic content of the wastewater since GNT antibiotic was not soluble in the wastewater containing PAHs. Although GNT is soluble in water, the solubility of GNT decreases significantly in waters containing benzene, toluen and PAHs [16]. Sınce low COD, GNT and BaP yields were obtained in aerobic activated sludge process, in this study, firstly, it was aimed to investigate the anaerobic degradability of BaP with high yields in the presence of GNT antibiotic using a high rate AMCBR. The AMCBR has many advantages compared to the other reactors such as a simple design due to no special gas or sludge separation, lower sludge generation, longer biomass and lower hydraulic retention times and higher stability to organic, hydraulic shock loads [17]. BaP and GNT were synthetically biodegraded in the AMCBR in order to determine the anaerobic biodegradation of BaP and GNT in single and dual substrate growth systems with and without primary substrates. The optimum HRTs and SRTs in the AMCBR were obtained from the preliminary studies [5, 18]. The removal and inhibition kinetics of BaP and GNT were evaluated. Furthermore, the raw petrochemical industry wastewaters were treated anaerobically in the AMCBR at varying BaP, GNT and COD concentrations originating from the industrial process, from the toilets and from the medical wastes.

1.1. Physico-Chemical Properties of BaP and GNT

The molecular weights of GNT and BaP were 477.6 and 252.31 g, respectively. Although GNT antibiotic is soluble in water, methanol and ethanol (6.67 mg/ L), its solubility is low in benzene PAHs (Table **1**). Although BaP is a hydrophobic compound, the solubility of BaP is high (0.00147 mg/ L) compared to GNT antibiotic (0.005 mg/ L) in the solution of hydrophobic polyhydrocarbons at 25 ^{o}C. An estimated Henry's Law constant of $8.9x10^{-7}$ kPa-m^3 /mol GNT in hydrophobic PAH solutions indicates that volatilization of GNT is not expected to be an important fate process. GNT's pKa of 4.89 indicates that it will exist predominately in the ionized form under environmental pH. Biodegradation of GNT from wastewater containing polyaromatic hydrocarbons is not expected to be an important fate process based on this compound for its estimated low Henry's law constant. As aforementioned, although BaP is a more hydrophobic PAH **(**Henry's law constant of BaP is $4.65x10^{-5}$ kPa-m^3/mol), the Henry's law constant of GNT remained lower than the BaP in petrochemical industry wastewaters indicating the lower degradability compared to BaP. The biodegradation abilities of PAHs and antibiotics depend on the physical and chemical properties of these compounds.

The EC_{50} value of BaP was measured as 156 mg L^{-1} to nematode *Caenorhabditis elegans* for prolonged exposure (72-h) at concentrations varying between 1 and 500 mg/L [19]. The 48-h EC_{50} for BaP was 174 µg/L with *Caenorhabditis elegans* at BaP concentrations varying between 10 and 500 µg/L. This was similar to that reported for *Daphnia magna* (200 µg/L). The EC_{50} value of GNT on *Staphylococcus aureus* was measured as 1 µg/L and 3 mg/L, depending on its concentrations [20]. It was mentioned that the EC_{50} values of GNT (1-11 mg/L and 0.1-12 µg/L) were 9.93 mg/L and 1.2 µg/L using *Escherichia coli.* The EC_{50} values obtained from the references given above showed that the GNT is approximetaly 20 times more toxic than BaP [21].

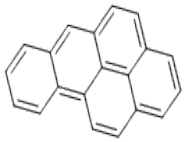
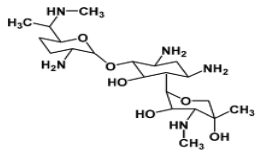
1.2. Kinetic Background

For batch biodegradation, biomass growth can be described by Eq. (1) [22], which can describe biomass growth due to single or dual substrates.

$$\frac{dx}{dt} = \mu X \qquad \text{Eq. (1)}$$

Depletion of growth associated substrates in a batch degradation for a given substrate, i, can be described using Eq. (2) [23].

Table 1: Physical-Chemical Properties of BaP and GNT

Physical-chemical properties	BaP[1]	GNT[2]
Molecular formula	$C_{20}H_{12}$	$C_{21}H_{43}N_5O_7$
Molecular Structure		
Molecular Weight (g mol^{-1})	252.31	477.6
Solubility in water (mg L^{-1})	$3x10^{-7}$	6.67
Solubility in hydrophobic solution (mg L^{-1})	0.00147	0.005
Henry's Law constant (kpA.m^3 mol^{-1})	$4.65x10^{-5}$	8.9×10^{-7}
logKow	6.20	-2.98
log Koc	4.0 to 8.3	Not available
Meltıng point (^{0}C)	177-180	218-237
Boiling point (^{0}C)	495	Not available
Density (g cm^{-3}) (at 25 ^{o}C)	1.351	1.302
Vapour density	8.7	Not available
Vapour pressure (mPa)	$0.37x10^{-6}$	Not applicable

logKow:Octanol/water partition coefficient; log Koc:Organic carbon partition coefficient [1, 23].

$$\frac{dS_i}{dt} = \frac{\mu_i X}{Y_x / S_i} \quad \text{Eq. (2)}$$

These equations hold true when maintenance requirements are negligible, which is typically assumed during the period of kinetic measurement of rapidly growing cells, as the metabolism of substrate is primarily growth associated [24]. It should be noted that the yield coefficient (Eq.2) must consider the consumption of compounds from both gas and liquid phases due to the volatility of BaP and GNT, assuming that transfer between gas and liquid phases is rapid. There are several models used to describe the specific growth rate for use in Eq.1 and Eq.2. The most common model for the biodegradation of a single growth substrate, the Monod model, is shown as Eq. (3) [24].

$$\mu_i = \frac{\mu_{\max i} S_i}{K_{S_i} + S_i} \quad \text{Eq. (3)}$$

Single substrate degradation experiments can be used to estimate the kinetic parameters mmax and K_S for each substrate. One method of estimating the kinetic parameters μmax and K_S involves fitting Eq. (3) to the experimentally obtained specific growth rates as a function of substrate concentration for single substrate experiments. Due to the toxic nature of BaP and GNT, the possibility of substrate inhibition was described with a modified Monod model, the Andrews model, as shown in Eq. (4) that may provide a better fit to experimental data obtained from single substrate experiments [25].

$$\mu_i = \frac{\mu_{\max i} S_i}{K_{S_i} + S_i + S_i^2 / K_I} \quad \text{Eq. (4)}$$

Again, the experimentally obtained specific growth rates can be plotted as a function of substrate concentrations and used in Eq. (4) to estimate the three kinetic parameters, μ_{max}, K_S and K_I. The kinetic parameters mmax, K_S and possibly K_I, which were determined from single component degradation processes, can be retained and used in specific growth rate models in which more than one growth limiting substrate is present. However, as stated previously, there is increased complexity in modeling multiple substrate degradation due to substrate interactions. Different models to describe the specific growth rate during the degradation of multiple interacting substrates have been developed in analogy to enzyme kinetics. The analogy can be made between enzyme kinetics and cellular kinetics because if a reaction is enzyme catalyzed, then the inhibition of enzyme

activity results in the inhibition of microbial growth by the same pattern. The models used to account for these interactions can be used in substrate degradation equations (Eq.2). A common interaction for BaP and GNT compounds is competitive inhibition, which can be seen in Eq. (5). During competitive inhibition, substrates compete for binding sites in order to be metabolized by the bacterial population.

$$\mu_i = \frac{\mu_{\max i} S_i}{K_{S_i}(1+(S_i / K_{S_i})) + S_i} \quad \text{Eq. (5)}$$

Another inhibition interaction is non-competitive inhibition where a nonreactive complex is formed when both substrates simultaneously are bound to one enzyme. This is shown as Eq.(6).

$$\mu_i = \frac{\mu_{\max i} S_i}{(K_{S_i} + S_i)(1+(S_i / K_{S_i}))} \quad \text{Eq. (6)}$$

Uncompetitive inhibition is another interaction that can occur when multiple substrates are present, which is shown in Eq. (7). Uncompetitive inhibition is a situation in which one substrate can bind to only a substrate-enzyme complex, not just the free enzyme.

$$\mu_i = \frac{\mu_{\max i} S_i}{K_{S_i} + S_i(1+(S_i / K_{S_i}))} \quad \text{Eq. (7)}$$

In Haldane inhibition, kinetic μ is usually described by Eq.(8) as follows

$$\mu_i = \frac{\mu_{\max} S}{K_S + S + \frac{S^2}{K_I}} \quad \text{Eq. (8)}$$

Where μ_{max}= maximum specific growth rate (1/h); K_S= half-velocity concentration (mg/ L), and K_I= inhibition constant (mg/ L). A higher value of K_I means a less inhibitive substrate. As the value of K_i approaches infinity, Eq. 8 reduces to the Monod equation.

2. MATERIALS AND METHODS

2.1. Reactor Configuration

The aboratory scale continuously fed stainless steel AMCBR used for the experimentation. The properties of AMCBR reactor, sludge source and nutrient properties were given in the previous studies [26].

2.2. Operational Conditions for AMCBR

The AMCBR was feed in continuous mode with and without molasses, GNT and BaP. The operational conditions for primary substrate, acclimated biomass, and GNT and BaP concentrations are given in Table **2**.

2.3. Analytical Methods

The COD in the influent and effluent samples was determined by a closed reflux colorimetric method [27]. The mixed liquor suspended solid (MLVSS) in the batch reactors and the VSS in the AMCBR were measured by a membrane filtration technique following standard methods [27]. For BaP, cis-4-(7-hydroxypyren-8-yl)-2-oxobut-3enac-acid, BaP-7,8-epoxide, benzo[*a*]pyrene-7,8-dihydrodiol, (+)benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide analyses; the water samples were filtered through a glass fiber filter (47 mm) to collect particle-phase in series with a resin column to collect dissolved-phase BaP. Resin and water filters were ultrasonically extracted for 60 min with a mixture of 1:1 acetone: hexane. All extracts were analyzed for BaP with a GC (Agilent 7890N) equipped with a mass selective detector (Agilent 5975 inert MSD). A capillary column (HP5-MS, 30 m, 0.25 mm, 0.25 m) was used. The initial oven temperature was held at 50 ^{0}C for 1 min, then raised to 200 ^{0}C at 25 ^{0}C/min and from 200 to 300

Table 2: Operational Conditions for BaP and GNT in the AMCBR

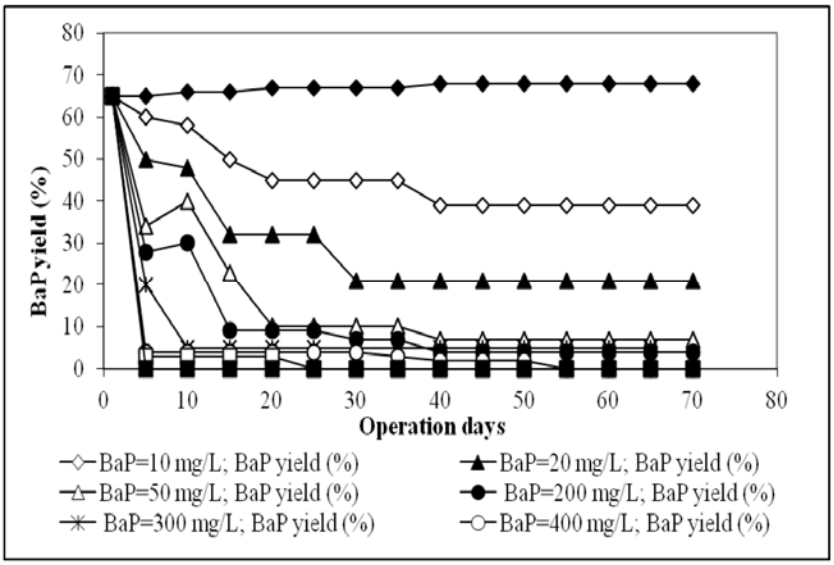
Conditions	Operation days	Primary substrate	BaP (mg L^{-1})	GNT (mg L^{-1})	Sole or mixing substrate
1	70	COD	-	0.01-100	Sole
2	70	COD	10-900	-	Sole
3	40-70	COD	400	50	Mixing
4	40-80-100	without	10-400	50	Mixing
5	70	without	-	0.01-100	Sole
6	30	without	10-900	-	Sole

^{o} C at 8 ^{0}C/min, and was held for 5.5 min. High purity helium was used as the carrier gas at constant flow mode (1.5 ml min^{-1}, 45 L/ cm linear velocity). BaP was identified on the basis of their retention times, target and qualifier ions and were quantified using the internal standard calibration procedure [11]. GNT and sisomicin, gentamine C1 and C2 measurements were carried out using an HPLC. Initially, all samples were centrifuged in the centrifuge (SED 5X model) to remove any particulate matter and then filtered through a 0.45 µm pore sized Teflon filter using a disposable syringe (Agilent 5185–5835) prior to HPLC analysis. Elution was prepared with isocratic solvent system consisting of (95:5%) methanol and formic acid. Thereafter, it was run at a flow-rate of 2.5 ml/min. The autosampler was set for an injection volume of 10 µl. The chromatographic separation of the sample was performed at 25^{o}C [5]. Detection was performed at 287 nm wavelength using an UV detector.

3. RESULTS AND DISCUSSION

3.1. Single Substrate Biodegradation of BaP without Primary Substrate in Unacclimated Anaerobic Bacteria

The BaP concentrations were adjusted from 10 mg/L up to 900 mg/L at a SRT and a HRT of 98 and 1.5 days in the AMCBR, respectively (Figure **1a**). 10 and



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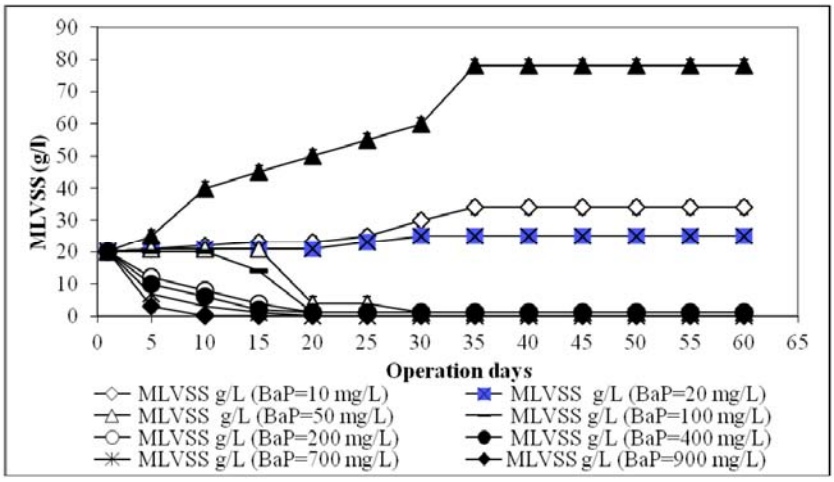

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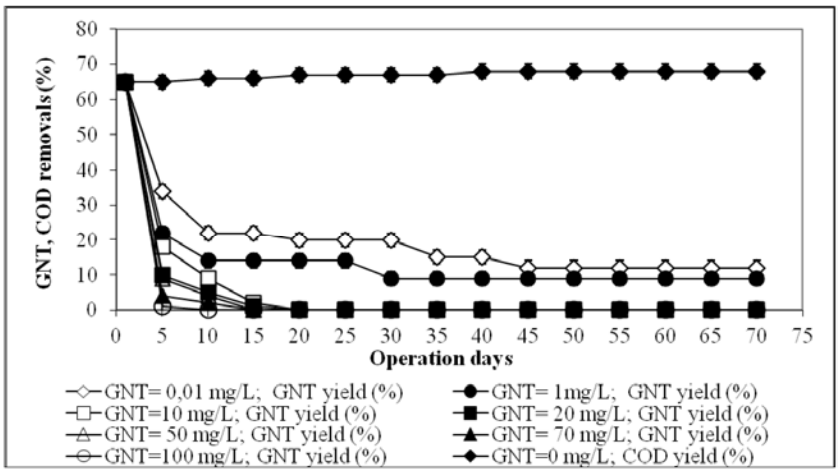
Figure 1: a: Variation of BaP yields versus increasing BaP concentrations in AMCBR without primary substrate in unacclimated anaerobic biomass

b: Variation of MLVSS concentration versus increasing BaP concentration in AMCBR without primary substrate in unacclimated anaerobic biomass.



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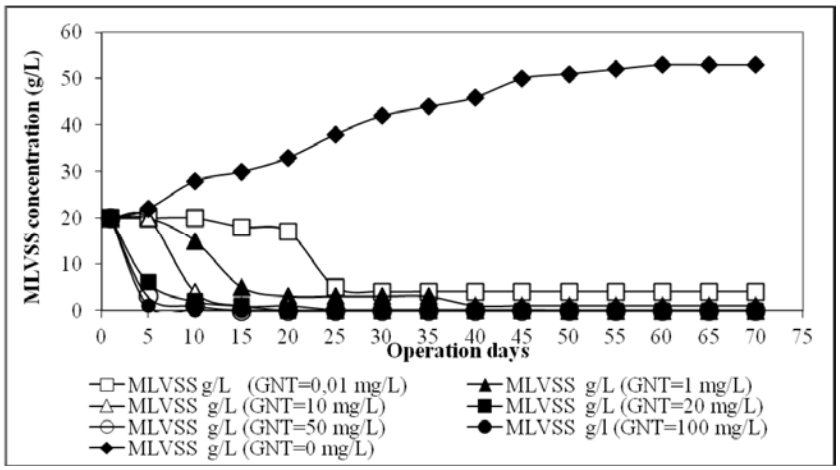

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Figure 2: a: GNT removals versus increasing GNT concentration in AMCBR without primary substrate in unacclimated anaerobic biomass.

b: Variation in MLVSS concentrations in AMCBR versus increasing GNT concentration in AMCBR without primary substrate in unacclimated anaerobic biomass.

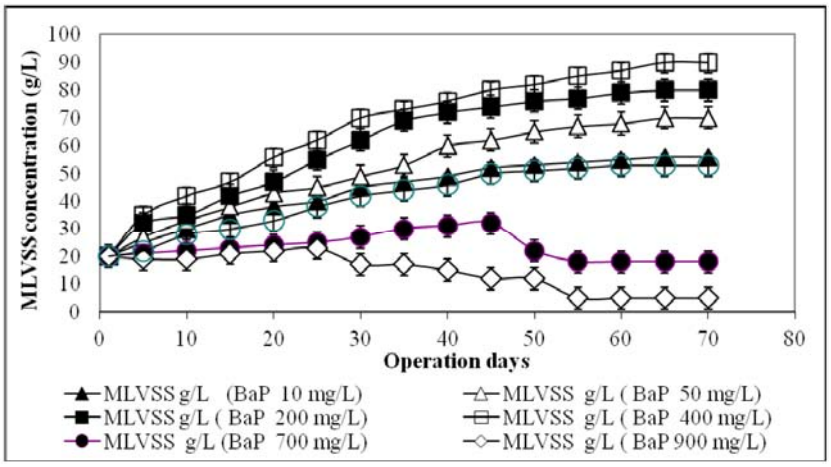
20 mg L^{-1} BaP was biodegraded with yields of 45% and 32% until days 35 and 24, respectively. The yields decreased to 39% and 21% after day 45 and day 35 of operation for BaP and GNT degradations, respectively. With the increase of initial BaP concentration, the final biomass decreased significantly, since high substrate concentration brought about strong substrate inhibition, which was quantifically demonstrated from the graph of cell growth and from the substrate degradation curve (Figure **1b**). Figure **2a** shows the GNT biodegradation profile at 0.01, 1, 10, 20, 50, 75 and 100 mg L^{-1} concentrations. The removal efficiencies in 0.01 and 1 mg L^{-1} GNT concentrations were 9% and 12% after 67 days of operation, respectively, in the AMCBR. 10, 20, 50, 75 and 100 mg L^{-1} GNT was not degraded. The MLVSS and GNT degradation rates were much lower in AMCBR than in BaP (see Figures **1a, 1b and 2a, 2b**), which indicated that GNT imposed the stronger substrate inhibition on the microorganisms. It was noticed that the final biomass yield in AMCBR containing GNT was smaller than that in the AMCBR containing BaP. The following two reasons were responsible for this result. GNT inhibition on cells was stronger than BaP, which resulted in the fact that low GNT consumption was used to overcome the substrate inhibition, and to synthesize new cells. This was due to the high acute toxicity of GNT compared to BaP, and some physicochemical properties of GNT.

3.2. Biodegradation Behavior of BaP without GNT in the Presence of Primary Substrate in Anaerobic Unacclimated Biomass

The AMCBR was operated at an initial molasses-COD concentration of 1460 mg/L and increasing BaP from 10 mg/L up to 900 mg/L at a SRT and a HRT of

98 and 1.5 days without GNT. The MLVSS concentration increased from initial 20 g/L up to 56 g/L after 67 days of operation period at a BaP of 10 mg/L (Figure **3a**). The slope of the log graph clearly demonstrated a gradual decrease of the growth of the control reactor with a final MLVSS concentration of 56 g/L. The MLVSS concentrations decreased in the BaP of 700 and 900 mg L^{-1} compared to the low BaP concentrations (Figure **3a)**. The MLVSS concentration increased from initial 20 g/L up to 32 g/L and 23 g L^{-1} on days 45 and 25, respectively, and then decreased probably due to the accumulation of BaP resulting in toxicity of the biomass (Figure **3b**). At the highest BaP concentration (900 mg /L), the MLVSS decreased sooner at short retention times (on days 25) in the control reactor compared to the AMCBR containing low BaP since the high BaP concentrations caused inhibition to microorganisms at short times. The COD yields increased from 67% up to 94%, after 67 days, at a BaP concentration of 200 mg/ L while the COD yield was 97% in the AMCBR containing 400 mg/ L BaP after 67 days. The anaerobic archael cells utilized the BaP as a secondary co-substrate together with molasses-COD as a primary carbon substrate for growth and energy.

In order to investigate how the unacclimated biomass in a specific environment can enhance the degradation of GNT, AMCBR was operated continuously throughout 67 days to achieve a sufficient contact between GNT, carbon source and microorganisms. Sufficient elimination was achieved for COD during the 67 days of continous operation at low GNT concentrations (0.01-50 mg/ L). This indicates that the biomass adapted to the low GNT concentrations (Figure **4a**). Under these conditions the



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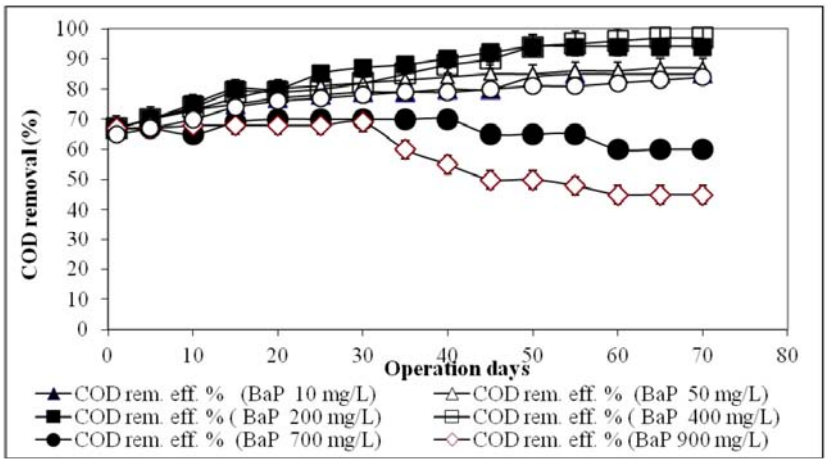

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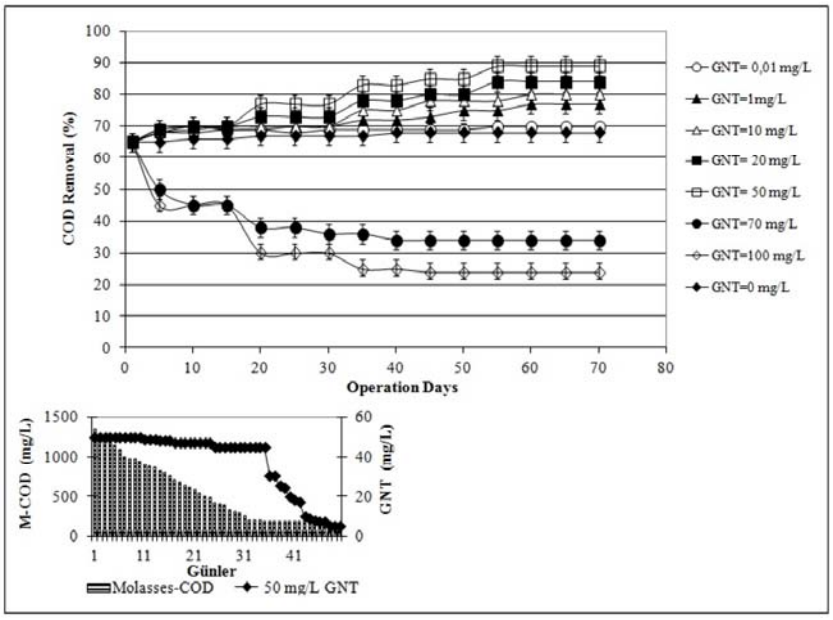
Figure 3: a: Effect of increasing BaP concentrations on MLVSS concentration in AMCBR in the presence of molasses-COD in anaerobic unacclimated biomass.

b: Effect of increasing BaP concentrations on COD yields in AMCBR in the presence of molassea-COD in anaerobic unacclimated biomass.



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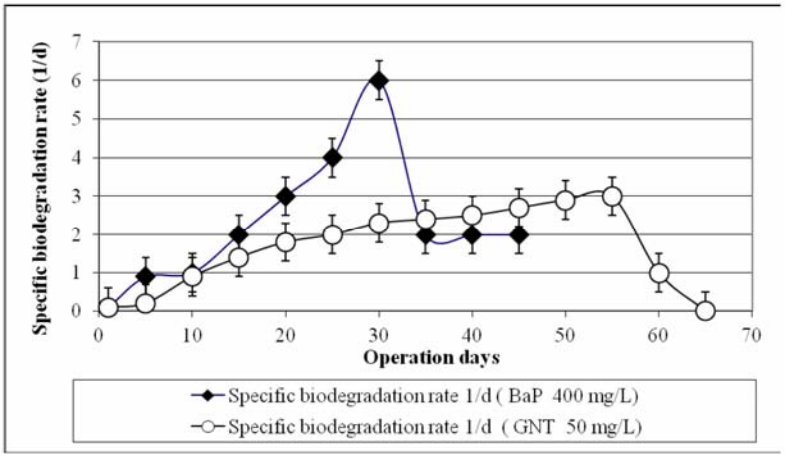

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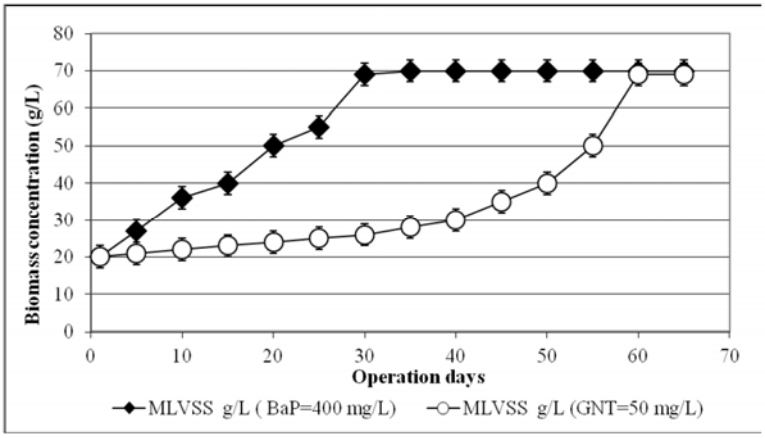


c

Figure 4: a: Effect of increasing GNT concentrations on COD removals in AMCBR in the presence of molasses-COD in anaerobic unacclimated biomass.

b: Variation of specific biodegradation rates in AMCBR for 400 mg L^{-1} BaP and 50 mg L^{-1} GNT in the presence of primary substrate in unacclimated anaerobic biomass.

c: Biomass concentration in AMCBR in the presence of molasses-COD, 400 mg L^{-1} BaP and 50 mg L^{-1} GNT in unacclimated anaerobic biomass.

dominated biomass can allow to the production of specific enzymes enable the degradation of this antibiotic at low doses. The COD yields were recorded as 70%, 77%, 80%, 84% and 89% in the AMCBR containing 0,01, 1, 10, 20, 50 mg /L GNT concentrations, respectively, after 67 days of the operation period indicating that low GNT concentrations can be used as an additional carbon source (Figure **4a**). The COD yield in AMCBR was measured as 68% for 67 days of operation period. The COD yields decreased for high GNT concentrations at the end of operation days. The concentration-time data for anaerobic *archae* biomass growth on the full molasses-COD and GNT substrate mixture is as follows: molasses-COD consumption started first and this compound was the first to be depleted (small Figure, inside Figure **4a**). 50 mg/L GNT consumption began later and degraded after 35 days of molasses-COD degradation ended (small Fig. inside Figure **4a**).

3.3. Biodegradation Behavior of GNT and BaP Mixtures in the Presence of Primary Substrate in Unacclimated Anaerobic Biomass

The maximum GNT biodegradation occurred at the initial GNT concentration of 50 mg/L. 400 mg/L BaP was almost completely degraded within 30 days; however, it took much longer (65-70 days) to consume the 50 mg/L GNT (Figure **4b**). A much higher specific biodegradation rate (6 /day) was observed in BaP, which indicated that GNT (maximum specific biodegradation rate = 3 /day) imposed a stronger substrate inhibition on the biomass after the BaP degradation terminated (after 35 days of operation). From the graph of cell growth, it is also clear that not only was the final biomass yield in AMCBR containing GNT smaller than that in AMCBR containing BaP, but also specific growth rates were lower in AMCBR containing GNT, as shown in Figure **4b**. The biomass concentration reached 73 g/L on day 29 in AMCBR, degrading 400 mg/L BaP while a retardation (after 60 days) was observed in AMCBR degrading 50 mg/L GNT to reach the maximum MLVSS concentration (69-70 g/L) (Figure **4c**).

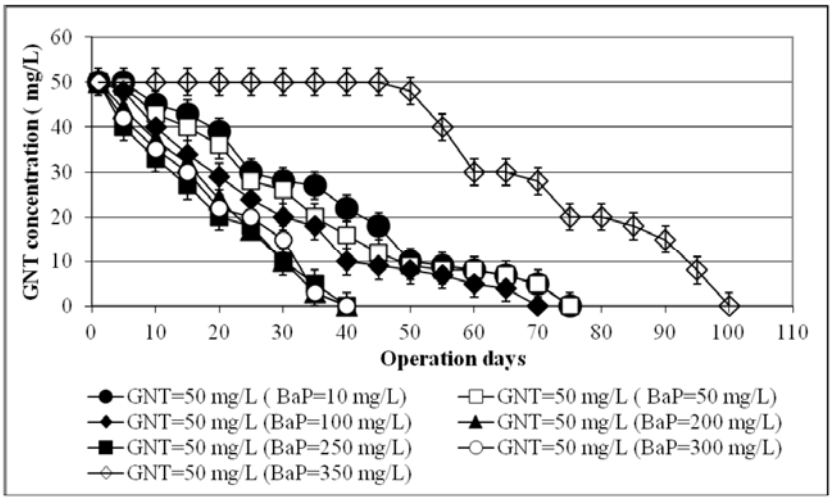
3.4. The Effect of BaP on GNT Biodegradation without Primary Substrate in Acclimated Biomass

The effects of increasing initial BaP concentrations from 10 to 400 mg/L on GNT biodegradation in the fixed GNT concentration of 50 mg/L in the AMCBR was investigated. It could be seen that the existence of a low-concentration of BaP inhibited the anaerobic GNT biodegradation (Figure **5a**). 50 mg/L GNT decreased to zero in a short time (on day 40) in the presence of 250 and 300 mg/L BaP while 50 mg/L GNT retarded to completely mineralized BaP (on day 70-72) when the BaP concentrations were between 10 and 100 mg/L. In the presence of low BaP concentrations, the GNT biodegradation increased until the BaP concentration was raised up to 350 mg/L (Figure **5a**). BaP, as a growth-substrate, supplied a carbon and energy source for biomass and was easily utilized to synthesize the new bacterial cells by the GNT degrading biomass. The bacterial cells grown in the AMCBR containing 50 mg/L GNT and BaP concentrations, increasing from 100 to 350 mg /L, exhibited logarithmic stages of the growth curves (see Figure **5b**). When BaP concentration increased beyond 400 mg /L, the inhibition increased with the increase of the initial BaP concentration resulting in a decrease in the biomass concentrations degrading 50 mg/L GNT. In spite of that, the biomass in the initial and the last stages of the biodegradation was still higher than that in the control reactor (BaP= 0 mg/ L) (Figure **5b**). With the further increase of initial BaP up to 700 and 1000 mg/L, the toxic property of BaP played a key role. MLVSS concentration reached 72, 77 and 90 g/L at BaP concentrations of 400, 200 and 300 mg /L, respectively, while the MLVSS were recorded as 70, 71 and 69 g/L for AMCBR containing 50 mg /L 100 and 700 mg /L BaP concentrations, respectively (Figure **5b**).

3.5. Kinetic Studies

Results from the continuous experiments in AMCBR containing only GNT or BaP in the presence of molasses-COD as the primary substrate were used to estimate the kinetic parameters. 20 and 50 mg/L GNT antibiotic and 200 and 400 mg/L BaP were removed following the Monod kinetic (Eqs. 1, 2, 3) in the presence of 1460 mg/L molasses-COD and unacclimated biomass. In this study, the kinetic coefficients relevant to primary substrate molasses-COD were not calculated (see Table **3**, conditions 1 and 2).

The maximum degradation rates i.e., μ_{max} and K_s for GNT in Monod Kinetic were 5.79 and 6.02 1/d, and 6 mg /L and 10 mg /L at 20 and 50 mg/L GNT concentrations, respectively (see Table **3**, condition 1). The yields and specific substrate utilization rates (SSUR) of *archae* biomass were 0.09, 0.11 unitless and 4.22 and 4.81 g GNT removed/g MLVSS.d at 20 and 50 mg/L GNT concentrations, respectively. The K_S and μ_{max} values in Monod kinetic for 200 and 400 mg/L



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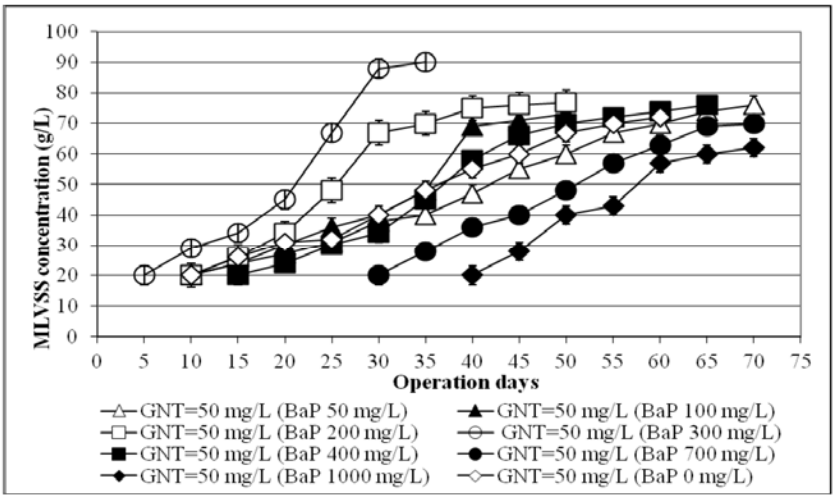

b

Figure 5: a: The effect of increasing BaP concentration on GNT biodegradation for 50 mg L^{-1} GNT in AMCBR without molasses-COD in acclimated biomass.

b: The effect of increasing BaP concentrations on MLVSS concentrations in AMCBR treating 50 mg L^{-1} GNT without molasses-COD.

BaP concentrations were calculated as 2 mg/L & 3 mg/L, and 8.67/d & 8.32/d, respectively. The SSURs and Y values were measured as 6.90 g BaP removed /g MLVSS.d, 6.21 g BaP removed /g MLVSS.d and 0.20 and 0.15 (unitless) (see Table **3**, condition 2). The K_S values for BaP were lower than K_S value for GNT antibiotic suggesting that the enzymes involved have a somewhat greater affinity for BaP than for GNT, as also previously observed that most of the isolated bacteria are able to grow on BaP as reported for low GNT concentrations such as 0.01, 1 and 10 mg/L; GNT was biodegraded by *archae* biomass according to non-competitive inhibition kinetic (Eq. 6) (see Table **3**, condition 1). The μ_{max} and K_S values were measured as 0.55 and 0.59/d, and as 0.005 mg/L and 9 mg/L at GNT concentrations of 0.01 and 10 mg /L, respectively. It was found that the K_S values did not change significantly based on the initial GNT concentrations while the μ_{max} values decresed significantly, at non-competitive inhibition kinetic for GNT antibiotic. The SSUR and Y values decreased at low GNT concentrations compared to the 20 and 50 mg/L GNT concentrations. K_I values were measured as 7 and 13 mg/L for the aforementioned GNT concentrations.

At high GNT concentrations (70 and 100 mg /L), the high GNT levels compete with molasses-COD to bind to the active centre of the enzyme or bind to a different site on the enzyme under competitive inhibition (Eq.5). In this inhibition type, the K_I values decreased indicating the high degree of inhibition with high K_S and SSUR values (Table **3**, condition1). The μ_{max} and

Table 3: Anaerobic Substrate Degradation and Inhibition Kinetic Results for the Different Conditions in the AMCBR Relevant to BaP and GNT

Condition 1							
The presence of primary substrate and unacclimated biomass, GNT biodegradation After 70 days							
GNT dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
0.01	69	NCI	0.55	3.77	0.005	0.01	7
1	75	NCI	0.56	3.79	0.08	-	-
10	79	NCI	0.59	4.01	9	0.02	13
20	84	Monod	5.79	4.22	6	0.09	-
50	89	Monod	6.02	4.81	10	0.11	-
70	35	CI	5.21	4.32	55	0.005	3
100	25	CI	5.70	4.78	80	0.001	4
Condition 2							
The presence of primary substrate and unacclimated biomass, BaP biodegradation After 70 days							
BaP dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
10	82	NCI	2.78	3.22	3	0.09	24
50	85	NCI	2.67	2.43	3	0.03	34
200	95	Monod	8.67	6.90	2	0.20	-
400	92	Monod	8.31	6.21	3	0.15	-
700	60	CI	7.78	2.19	245	0.004	9
900	45	CI	7.89	1.99	467	0.001	6
Condition 3							
The presence of primary substrate and unacclimated biomass, mixture of 50 mg/L GNT After 70 days and 400 mg/L BaP After 40 days							
BaP dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
400	93	Monod	9.01	7.12	2	0.23	-
GNT dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
50	80	Monod	7.01	6.56	9	0.18	-
Condition 4							
Without primary substrate, with acclimated biomass, effect of increasing BaP concentration on biodegradation of 50 mg/L GNT							
BaP Dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
10	100 (80 days)	NCI	3.02	2.67	13	0.12	9
50	100 (80 days)	NCI	3.04	2.71	13	0.13	12
100	100 (70 days)	NCI	3.04	2.72	15	0.14	18
200	100 (70 days)	NCI	3.12	2.79	11	0.14	21
250	100 (40 days)	Monod	4.98	2.89	9	0.28	-
300	100 (40 days)	Monod	5.01	2.97	7	0.29	-
350	100 (100 days)	CI	3.01	1.89	129	0.11	3
400	100 (100 days)	CI	2.99	1.93	230	0.09	4

(Table 3). Continued.

Condition 5							
GNT biodegradation as single substrate without primary substrate and acclimated substrate After 70 days							
GNT dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
0	89	Monod	3.99	3.56	10	0.29	-
0.01	20	NCI	1.19	1.98	8	0.14	
1	10	CI	3.23	1.78	23	0.12	
10	0,5	HI	0.0001	0.002	23	0.0001	1
20	0,5	HI	0.0001	0.002	45	0.0001	1
50	0,3	HI	0.0001	0.002	49	0.0001	0.1
70	0,2	HI	0.0001	0.002	69	0.0001	0.01
100	0,2	HI	0.0001	0.002	97	0.0001	0,01
Condition 6							
BaP biodegradation as single substrate without primary substrate and acclimated substrate After 30 days							
BaP Dose	**Removal (%)**	**Kinetic**	**μ_{max}**	**SSUR**	**K_S**	**Y**	**K_i**
10	43	Monod	2.01	1.38	4	0.56	-
20	21	CI	1.71	1.30	40	0.21	15
50	10	CI	1.60	1.29	75	0.06	20
300	5	CI	1.57	1.23	670	0.02	22
400	5	CI	1.34	1.21	800	0.02	34
500	0.9	HI	0.99	0.0004	700	0.001	0.01
700	0.7	HI	0.99.	0.0003	900	0.001	0.01
900	0.2	HI	0.56	0.0001	1560	0.001	0.01

NCI: non-competitive inhibition, HI:Haldane inhibition, CI: competitive inhibition [29, 31].

SSUR levels increased more than that of non-competitive inhibition. The high K_S values were accomplished due to the low affinity of anaerobic *archae* to GNT. This results in lower yield values as compared to non-competitive inhibition.

At low BAP concentrations such as 10 and 50 mg/L, BaP was biodegraded by *archae* biomass according to the non-competitive inhibition kinetic (Table **3**, condition 2). The μ_{max} and K_S values were measured as 2.78/d and 2.67/d, and as 3 mg/L and 3 mg/L, respectively, at the low BaP concentrations. For BaP concentrations between 200 and 400 mg L^{-1}, BaP was biodegraded according to Monod kinetic. The μ_{max}, SSUR and Y values were at the highest levels. No significant BaP accumulation was observed with lowest K_S values in Monod kinetic for the BaP concentrations.

At high BaP concentrations (700-900 mg /L), competitive inhibition is mainly predominant (Table **3**, condition 2). In this type of inhibition, the K_S values increased significantly compared to Monod and non-competitive inhibitions, while the Y and SSUR values decreased. The K_i values also increased compared to non-competitive inhibition hence indicating the high inhibition at high BaP concentrations.

Some substrate interaction affects the biodegradation of PAHs by pure and mixed cultures. Sometimes, high molecular weight PAHs have been utilized/degraded after the PAHs with low molecular weights were biodegraed [28,29]. For example, high concentration of naphthalene may have inhibited the degradation of other PAHs due to its toxicity [28]. Stringfellow and Aitken found competitive inhibition of phenanthrene on the degradations of naphthalene, methylnaphthalene, and fluorene in binary mixtures using two pure cultures [28]. They concluded that the occurrence of competitive inhibition observed with two different pseudomonas species might be common among PAH-degrading organisms. The presence of

phenanthrene is reported to inhibit degradation of pyrene [29]. In studies with pure denitrifying isolates, the presence of naphthalene enhanced both phenanthrene and pyrene degradation, whereas phenanthrene apparently inhibited pyrene degradation.

Some studies have even reported the stimulation of degradation of PAHs when present in mixtures. The biodegradation of PAHs in varying mixture combinations by pure culture of *Pseudomonas putida* strain KBM-1, under aerobic conditions, showed that the presence of naphthalene (2-ringed PAH) stimulated phenanthrene (3-ringed PAH) degradation 5-fold and pyrene (4-ringed PAH) degradation 2-fold. It was reported that the degradation efficiency of microorganisms is more vigorous when acenaphthene, fluorene, phenanthrene, anthracene, and pyrene are present simultaneously compared to the rate of degradation when the PAHs are present individually because the presence of all five compounds provides more carbonsource, or cross acclimation may enhance the rate of biodegradation [30].

In the mixture of 50 mg/L GNT and 400 mg/L BaP concentration, 400 mg/L BaP was degraded before GNT at short times initially with high μ_{max}, SSUR, Y and low K_S values (in the presence of molasses-COD and unacclimated biomass (Table **3**, condition 3). The high affinity of BaP than GNT to anaerobic *archae* bacteria could be attributed to high solubility and high Henry's law constant of BaP ($4.65x10^{-5}$ kPa.m^3 /mol) than GNT ($8.9x10^{-7}$ kPa.m^3 /mol) in the solutions containing PAHs.

In the absence of primary substrate and in the presence of acclimated biomass, 50 mg/L GNT was completely biodegraded within 40 days with a yield of 100% at BaP concentrations of 250 and 300 mg/L (Table **3**, condition 4). Under optimum BaP concentrations, there was a marked enhancement of GNT mineralization at short operation days compared to low BaP concentrations (10-200 mg/L). BaP was probably used as electron giving growth substrate under anaerobic conditions together with the GNT. Furthermore, it was probably used as a carbon and energy source together with the GNT. At low and high BaP concentrations, no significant differences in GNT removal efficiencies were observed. This could be explained by the time required to reach maximum GNT removal at low and high BaP levels. In other words, a delay was detected at low and high BaP concentrations. At low BaP concentrations (10-200 mg /L), competitive inhibition was detected. In this inhibition, the K_S and SSUR values were not significantly varied compared to Monod while the μ_{max} values decreased to 3.02-3.12 mg/L together SSUR levels. At high BaP concentrations (350-400 mg/ L), competitive inhibition predominated with an increase of K_S values and lowering in K_i values as compared to low BaP indicating the high inhibition compared to non-competivive inhibition.

In the absence of primary substrate and in unacclimated biomass, very low GNT yields were obtained (Table **3**, condition 5). In control reactor without GNT, the molasses-COD was biodegraded with a yield of 89% and a K_S value of 10 mg/L (Table **3**, condition 5). In Haldane inhibition kinetic (Eq.8), the μ_{max} values decreased and K_S values increased with high K_i values indicating the substrate inhibition. At 1 mg/L GNT concentration, the substrate was removed according to competitive inhibition. In this inhibition, μ_{max} and Y significantly decreased to 3.23/d and 0.12 unitless compared to Monod kinetic. For very low GNT concentration (0.01 mg/ L), the GNT was removed according to competitive inhibition kinetic. The μ_{max} and yield Y decreased to 1.19 /d and to 0.14 unitless, respectively. At high GNT concentrations (10-100 mg/ L), the substrate was removed according to Haldane kinetic. The μ_{max}, SSUR and Y values decreased significantly, while K_S increased. The inhibition constant K_i decreased as the GNT concentration increased indicating the inhibitory effect of high GNT concentrations.

In the absence of molasses-COD and unacclimated substrate, BaP was removed with a yield of 43% at a BaP concentration of 10 mg/L according to Monod kinetic. The μ_{max}, Y and K_S values were measured as 2.01/d, 0.56 and 4 mg/L, respectively. (Table **3**, condition 6). For BaP concentration between 20 and 400 mg/L, competitive inhibition was observed. In this inhibition, the μ_{max} was not decreased compared to the Monod kinetic as the BaP concentration was increased from 10 to 400 mg/L. Very strong inhibition was observed in Haldane substrate inhibition model with low K_i (0.01 mg /L). In this inhibition, the μ_{max} values decreased and the K_S values increased (Table **3**, condition 6).

3.6. BaP and GNT Metabolites under Anaerobic Conditions in AMCBR

During the anaerobic BaP and GNT biodegradation process, metabolites formed in different conditions were analyzed using GC-MS; in the absence of

molasses-COD when the BaP is used as sole substrate by the acclimated anaerobic bacteria, from 10 mg/L BaP 4.05 mg/L cis-4-(7-hydroxypyren-8-yl)-2-oxobut-3enac-acid was obtained as metabolite of BaP after 20 days of operation (Table **4**). Since the BaP removal efficiency is 43% (condition 6), the rest of BaP (10 mg /L - 4.3 mg/ L =5.7 mg /L) probably remained and could not be metabolized in AMCBR under anaerobic conditions. A large part of 4.05 mg/L cis-4-(7-hydroxypyren-8-yl)-2-oxobut-3enac-acid was mineralized under anaerobic conditions in AMCBR and it was reduced to 0.05 mg/L indicating that it metabolized to methane, carbondioxide and water after 70 days of operation (Table **4**). In the presence of molasses-COD as the primary substrate and unacclimated biomass, from 200 mg/L BaP, 70 mg/L benzo[*a*]pyrene-7,8-epoxide, 90 mg/ L (-)benzo[*a*]pyrene-7,8-dihydrodiol and 29 mg/L (+)benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide metabolites (condition 2) were produced on operation day 20 (Table **4**). Removal efficiency of 200 mg/L BaP was 95%, 10 mg/L BaP was not biodegraded and went into the effluent of AMCBR. Therefore, it can be concluded that 1 mg/L BaP was directly mineralized to methane gas, carbon dioxide and water without being converted to BaP metabolites on day 20. Since the metabolite concentrations decreased to 0.5, 0.05 and 0.1 mg/L for benzo[*a*]pyrene-7,8-epoxide, benzo[*a*]pyrene-7,8-dihydrodiol and (+)benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, respectively, after 70 days of the operation period it can be concluded that all the metabolites were mineralized [31]. 0.002 mg/L GNT was biodegraded under anaerobic conditions with a yield of 20% while the rest of GNT (0.008 mg/ L) was not degraded in the AMCBR. 0.002 mg/L GNT was metabolised to 0.0005 mg/L sisomicin and to 0.001 mg/L gentamine C1 (condition 5) when thr GNT was used as a single substrate by the acclimated anaerobic bacteria [32]. In the presence of primary substrate and unacclimated biomass, from 50 mg/L GNT, 44.5 mg L^{-1} GNT was metabolised to 25 mg L^{-1} Gentamicine C1 and to 18 mg/L Gentamine C2 after 20 days (condition 1). These metabolites were reduced to 1 mg/L and 0.05 mg/L, respectively, after 70 days by unacclimated anaerobic bacteria. This means that these metabolites mineralized to the the final products such as methane and carbondioxide while the rest of the GNT and metabolites (44.5 mg/ L^{-1} -(43 mg/ L) =1.5 mg/L and 1.05 mg/L, respectively, were released into the effluent. The anaerobic metabolites of the mixture of 400 mg/L BaP and 50 mg/L GNT were researched in the the presence of primary substrate and unacclimated biomass (condition 3). From 400 mg /L BaP, 345 mg/L benzo[*a*]pyrene-7,8-dihydrodiol was produced after 20 days of operation while from 50 mg/L GNT, only 20 mg/L gentamine C1 and 17 mg/L gentamicine C2 metabolites were produced. The BAP metabolite metabolized and its concentratıon was reduced to 0.2 mg/L on day 70. The rest of BaP (27 mg/ L) was not used by the unacclimated anaerobic bacteria [33].

4. DISCUSSION

In the presence of primary substrate and unacclimated bacteria to BaP and GNT for only certain BaP and GNT concentrations maximized the BaP and

Table 4: BaP and GNT Metabolites According to Studied Conditions

BaP and GNT concentrations	Yield	Day 20	Day 70	Condition
10 mg L^{-1} BaP	BaP%43	4.05 mg L^{-1} cis-4-(7-hydroxypyren-8-yl)-2-oxobut-3enac-acid	0.05 mg L^{-1} cis-4-(7-hydroxypyren-8-yl)-2-oxobut-3enac-acid	2
200 mg L^{-1} BaP	BaP%95	70 mg L^{-1} benzo[*a*]pyrene-7,8-epoxide, 90 mg L^{-1} (-)benzo[*a*]pyrene-7,8-dihydrodiol, 29 mg L^{-1} (+)benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide	0.5 mg L^{-1} benzo[*a*]pyrene-7,8-epoxide, 0.05 mg L^{-1} benzo[*a*] pyrene-7,8-dihydrodiol, 0.1 mg L^{-1} (+)benzo[*a*] pyrene-7,8-dihydrodiol-9,10-epoxide	6
0.002 mg L^{-1} GNT	GNT %20	0.0005 mg L^{-1} sisomicin, 0.001 mg L^{-1} gentamine C1	-	5
50 mg L^{-1} GNT	GNT %89	25 mg L^{-1} Gentamicine C1, 18 mg L^{-1} Gentamine C2	1 mg L^{-1} Gentamicine C1, Gentamine C20.05 mg L^{-1},	1
400 mg L^{-1} BaP and 50 mg L^{-1} GNT	Bap %93; GNT % 80	345 mg L^{-1} benzo[*a*]pyrene-7,8-dihydrodiol 20 mg L^{-1} gentamine C1 17 mg L^{-1} gentamicine C2	0.2 mg L^{-1} benzo[*a*]pyrene-7,8-dihydrodiol	3

Table 5: Variation of BaP, GNT and COD Degrading Bacteria Numbers in the Real Anaerobic Activated Sludge Reactor from the Real Petrochemical Industry Wastewater Treatment Plant

BaP degrading bacteria number (cfu ml^{-1})	GNT degrading bacteria number (cfu ml^{-1})	Heterotrophic bacteria number (cfu ml^{-1})	Operation days
$34x10^3$	$23x10^2$	$5x10^6$	1-6
60	60	$1x10^3$	18-26
$34x10^5$	$12x10^4$	$4x0^7$	62-80
$23x10^1$	$15x10^1$	$67x10^2$	83-105
78	45	$45x10^4$	108-120

GNT yields as was observed in the separate AMCBR within 70 days (for 200 mg L^{-1} and 400 mg/L BaP and 20 mg/L and 50 mg/L GNT). They were anaerobically removed with high yields as 92-95% and 84-89%, respectively, within 70 days. At low BaP (10, 50 mg/ L) and GNT concentrations (0.01-10 mg /L), molasses-COD was used as the primary substrate by the high concentrations of heterothrophic bacteria ($34x10^9$ cfu /ml) (Table **5**). At low BaP and GNT concentrations, the number of specific BaP degrading bacteria number was low ($23x10^2$ cfu /ml) and they did not compete effectively with the heterothrophic bacteria and, therefore, they did not biodegrade the BaP and GNT as much as the BaP and GNT concentrations given above (Table **5**). Under these conditions, low BaP and GNT yields were observed. A non-competitive inhibition was observed; therefore, low reaction rates were also observed resulting in low yields.

At high BaP (700-900 mg/ L) and GNT (70-100 mg /L) concentrations, due to acute toxicity of these substances, low removal efficiencies were measured. Since the acute toxicity (EC_{50}=9.93 mg/ L) values of GNT was high as compared to the BaP (EC_{50}=156 mg /L), lower GNT yields (25-35%) were observed than BaP [34,35]. The mixture of 50 mg/L GNT and certain BaP concentrations increased the GNT yields without the primary substrate and with acclimated biomass. 250 mg/L BaP shortened the retention time significantly for removing the 50 mg/L GNT with the same maximum yield (100%). The anaerobic degradability of single GNT as a sole substrate was low in the absence of primary substrate and in the presence of acclimated biomass. 20% maximum GNT yield was observed for 0.01 mg/L ^{of} GNT after 70 days of operation. The GNT yields decreased to nearly zero as the GNT concentrations were increased from 0.01 mg/L up to 100 mg/L. The maximum anaerobic biodegradability of 10 mg/L BaP, as the sole substrate, was only 43% without the primary substrate and with acclimated biomass within 30 days of operation. In both reactors, the effect of acclimated biomass on BaP and GNT yields was significantly higher than that of the unacclimated biomass (data not shown) (t test=2.43, p=0.05)

In the absence of primary substrate and in the presence of acclimated biomass, when BaP and GNT were used as the sole substrates, low BaP & GNT yields were obtained. However, it is important to note that a higher BaP yield was detected (43%) than that of GNT (20%). This can be attributed to the fact that GNT (EC_{50}=9.93 mg/L) was more toxic than BaP (EC_{50}=156 mg/ L) as it exhibited greater inhibitory effects on the acclimated cell growth behaviors. On the other hand, the Henry law constant of GNT ($8.9x10^{-7}$ kpA.m^3 /mol) in benzen and aromatic hydrocarbons solution was low as compared to the Henry law constant of BaP (**(**$4.65x10^{-5}$ kPa.m^3 /mol**)** hence indicating that the acclimated anaerobic biomass utilized more BaP than GNT [36]. In this run, both BaP and GNT were used as carbon and energy sources by the anaerobic bacteria.

In the absence of primary substrate and in the presence of acclimated biomass, the mixture of 50 mg/L GNT and 250 mg/L BaP shortened the retention time (40 days) required to reach maximum BaP and GNT degradations. Both GNT and BaP yields were 100%. GNT and BaP were completely mineralized. At BaP concentration of 10-200 mg/L, 100% BaP and GNT yields were observed. In mixtures of certain BaP and GNT concentrations, the BaP yields increased slightly compared to when BaP was used as the sole substrate in the presence of primary substrate and unacclimated biomass. The elevated degradation yield of GNT substrate was affected by the presence of a certain BaP concentration in condition 4. The K_S of GNT is regarded as the major prerequisite for achieving efficient and nearly complete GNT degradation. Moreover, the effect of BaP on the efficiency of GNT removal is strongly dependent on the

K_S-GNT/K_S-BaP ratio, consequently, determination of GNT and BaP degrading microbes is important when biodegradation is considered.

The competitive inhibition model predicts that the relative effect of BaP on the kinetics of GNT degradation increases with increasing ratios of K_S-GNT to /K_S-BaP**.** The relative importance of K_S-GNT /K_S-BaP ratio on the anaerobic biodegradation of BaP and GNT may also greatly depend on the studied conditions in AMCBR. In the mixture of BaP and GNT (condition 4), at high BaP concentrations (350-400 mg /L), K_S-GNT/K_S-BaP ratio increased from 64 mg/L up to 121 mg/L since 50 mg /L was not biodegraded and remained in the AMCBR. Under these conditions, a severe competitive inhibitory effect was observed, and the presence of microorganisms characterized by a high K_S-GNT /K_S-BaP ratio was determined. In contrast, in AMCBR with certain levels of BaP (10-200 mg/ L), the K_S-GNT/K_S-BaP ratio was around 5 and 6 mg/L. Under these conditions, a non-competitive inhibition was observed by a low K_S-GNT/K_S-BaP ratio since the K_S was not changed. When BaP and GNT were as sole substrates, at very high substrate concentrations, μ_{max} and K_I value significantly decreased. A severe inhibition was defined with Haldane kinetic. However, at high substrate concentrations, the inhibitory effects on cell growth were stronger than low substrate concentration, and also more energy was required to maintain the cell activity, but it may not have been enough to synthesize new cells. Another possible reason for the decreased cell mass was the production and accumulation of various intermediates such as cis-4-(7-hydroxypyren-8-yl)-2-oxobut-3enac-acid, benzo[*a*]pyrene-7,8-epoxide, 90 mg/ L benzo[*a*]pyrene-7,8-dihydrodiol and 29 mg /L (+)benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide and sisomicin and gentamine C1 as BaP and GNT intermetabolites.

5. CONCLUSIONS

The biomass concentration, BaP and GNT yields decreased with the increase of initial BaP and GNT concentrations because high BaP and GNT levels provided strong inhibitions in the AMCBR. This could be due to the fact that GNT is more toxic than BaP, based on the EC_{50} values, as is more soluble than BaP when it is in the composition of petrochemical industry wastewater containing solvents, PAHs and some hydrophobic organic substances. The reason for the low BaP and GNT yields at low GNT and BaP concentrations could be attributed to the insufficient specific biomass concentration, which would degrade the GNT and BaP in the absence of primary substrate. Under these conditions, with low bıomass, low BaP and GNT yields were obtained. BaP was degraded at short operation times compared to GNT. The operation time required to reach maximum MLVSS for GNT was double the time required for BaP. The reasons for this could be attributed to the higher acute toxicity values of GNT compared to BaP. Low BaP concentrations retarded the time required for complete GNT degradation. There is little information available on the occurrence and fate of GNT, a class of aminoglycosides, in wastewater and through treatment processes. In the presence of molasses-COD, a short lag phase was observed for high and low BaP and GNT concentrations. The inhibitory effects of BaP and GNT were clearly observed on the molasses-COD assimilation while BaP and GNT concentrations were increased. Molasses-COD was effectively consumed in the presence of BaP and GNT. At high BaP concentrations, competitive inhibition was detected with an increase in K_S values and decrease in K_I values when compared with the non-competivive inhibition. In the absence of molasses-COD; for BaP concentration between 20 and 400 mg/L, competitive inhibition was observed. In this inhibition, the μ_{max} was not decreased. Very strong inhibition was observed in Haldane substrate inhibition with low K_I and increasing KS values at high GNT concentrations.

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NOMENCLATURE

PAH = Polycyclic aromatic hydrocarbon

BaP = Benzo[a]pyrene

GNT = Gentamicin

AMCBR = Anaerobic multichamber bed reactor

USEPA = United States Environmental Protection Agency

HPLC/MS = High pressure liquid chromatograph/mass spectrometry

COD = Chemical oxygen demand (mg/ L)

HRT = Hydraulic retention time (d)

SRT = Solid retention time (d)

BOI_5 = Biochemical oxygen demand (mg/ L)

CSTR = Continuously stirred tank reactor

EC_{50} = Effective concentration (mg/ L)

VSS = Volatile suspended solid (m/ L)

TSS = Total suspended solid (mg/ L)

MLVSS = Mixed liquor volatile suspended solids (mg/ L)

GC/MS = Gas chromatograph/mass spectrometry

S = Substrate concentration (mg/ L)

SSUR = Specific substrate utilization rate (g substrate removed/ g MLVSS.d)

μ_{max} = Maximum specific growth rate (1/h)

K_S = Half-velocity concentration (mg/ L)

K_I = Inhibition constant (mg/ L)

Y = Yield coefficient (unitless)

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