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Performance of the hybrid growth sequencing batch reactor (HG-SBR) for biodegradation of phenol under various toxicity conditions

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ABSTRACT

Hybrid growth microorganisms in sequencing batch reactors have proven effective for treating the toxic compound phenol, but the toxicity effect under different toxicity conditions has rarely been discussed. Therefore, the performance of the HG-SBR under toxic, acute and chronic organic loading can provide the overall operating conditions of the system. Toxic organic loading (TOL) was monitored during the first 7 hr while introducing 50 mg/L phenol to the system. The system was adversely affected with the sudden introduction of phenol to the virgin activated sludge, which caused a low degradation rate and high dissolved oxygen consumption during TOL. Acute organic loading (AOL) had significant effects at high phenol concentrations (600, 800 1000 mg/L). The specific oxygen uptake rate (SOUR) gradually decreased to 4.9 mg O_2/(g MLVSS·hr) at 1000 mg/L of phenol compared to 12.74 mg O_2/(g MLVSS·hr) for 200 mg/L of phenol. The HG-SBR was further monitored during chronic organic loading (COL) over 67 days. The effects of organic loading were more apparent at 800 mg/L and 1000 mg/L phenol concentrations, as the removal range was between 22%–30% and 18%–46% respectively, which indicated the severe effects of COL.

Introduction

Phenol is considered to be a volatile organic compound which is capable of causing negative affects towards human health and creating a hazard to the environment (Cheng et al., 2016). The annual production of phenol, estimated at 10 million ton worldwide, has led to concern about the removal of this organic compound from wastewater (Yang et al., 2012). Organic compounds, whether from the industrial or domestic sector, are aerobically oxidized; and an aerobic treatment system is the most suitable wastewater treatment process. In aerobic processes, microorganisms that oxidize organic matter to produce carbon dioxide and water as a final product are known as heterotrophic bacteria. Apart from that, microorganisms that derive cell carbon from carbon dioxide are called autotrophic bacteria. The energy required for cell synthesis is obtained from the oxidation of the organic compounds. Heterotrophic bacteria oxidize the dissolved and particulate carbonaceous organic matter into simple by-

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products and additional biomass for aerobic biological oxidation (Metcalf and Eddy, 2004) as described by Eq. (1):

$$\text{Organic matter} + O_2 + NH_3 + PO_4^{3-} \xrightarrow{\text{microorganisms}} \text{new cells} + CO_2 + H_2O \quad (1)$$

Oxygen (O_2), ammonia (NH_3) and phosphate (PO_3^{4-}) are nutrients needed by microorganisms to convert organic matter to carbon dioxide (CO_2) and water. New cells represent the biomass produced as a result of the oxidation of the organic matter. Aerobic heterotrophic microorganisms are able to produce extracellular biopolymers that contribute to the formation of biological flocs (Metcalf and Eddy, 2004). This kind of behavior correlates to the basic principles of biological processes using either aerobic or anaerobic oxidation for wastewater treatment, which can be divided into two categories: the suspended growth process and attached growth (biofilm) process. The suspended growth process has been widely applied in municipal (Morgenroth et al., 1997; Aiyuk et al., 2006) and industrial wastewater treatment (Scott and Smith, 1995; Kantardjieff and Jones, 2000) in aerobic conditions; while, for anaerobic conditions, Zhou et al. (2018) studied the effect of Zn^{2+} on ammonium and phosphorus removal and duckweed growth in anaerobically digested swine wastewater.

For high concentration wastewater and organic sludge, the suspended process occurs under anaerobic conditions. However, the main drawback of the process is mainly related to mixed liquor inventory control and sludge wasting. Compared to the suspended growth process, mixed liquor is more stable in attached growth, with minimal sludge wasting. Microorganisms such as heterotrophic bacteria, particulate matter and extracellular polymers in the form of biofilm, can attach to and occupy the packing material in attached growth processes (Metcalf and Eddy, 2004). The substrates are consumed within the biofilm. Biofilm is a complex non-uniform structure with uneven protrusions. The liquid can flow through either vertical or horizontal pores. The biofilm formed is dense with biomass and varies in density and depth. Uniform growth of biofilm onto packing materials usually does not occur (Hinton and Stensel, 1991).

Thus, specifically for the aerobic process, a hybrid (combined) suspended and attached growth process is another option to be considered. The Fluidized Bed Reactor (Vinod and Reddy, 2006; Cisneros-Pérez et al., 2017; Di Capua et al., 2017), Expended Bed Reactor (Chu et al., 2005), Immersed Media Systems (Marrot et al., 2006; Qi et al., 2009; Cheng et al., 2014), and Porous Support Systems (Karthikeyan et al., 2015; Chen et al., 2017; Edathil et al., 2017) are examples of hybrid growth systems. The combination of the two processes creates a hybrid, providing the advantages of two individual processes. Organic compound removal could be achieved with the volumetric efficiency and low energy requirements of attached growth processes. The attached growth acts as a biological selector to improve activated sludge settling characteristics, while the activated sludge treatment has the capability to produce high quality effluent. Apart from that, the hybrid process can provide stability and resistance to shock loads for the whole treatment system.

Shock load is a crucial condition that may happen in any treatment system. Industrial effluents are prone to shock load, as a different process might suddenly produce a high concentration of wastewater. Due to this condition, it is crucial to evaluate the toxicity effects on a biological treatment system during shock load conditions. Kulkarni (2012) reported on the effect of shock loads on the simultaneous nitrification and denitrification (SND) of 4-nitrophenol, 2, 4-dinitrophenol and 2, 4, 6-trinitrophenol up to concentrations of 600 mg/L each. Moreover, a pseudo-steady state was achieved during mixed loading of all three compounds. Mizzouri and Shaaban (2013) analyzed the effects of toxic, hydraulic and organic shock on the performance of a lab-scale sequencing batch reactor for treatment of petroleum refinery wastewater.

Despite a number of research works on shock loads, there is no single study discussing the effect of toxicity under different conditions due to shock loads in the hybrid growth biological treatment system. Moreover, it is crucial to know the toxicity effect especially during the first time a toxic pollutant is introduced, after each change of concentration, and the long-term effect of shock loads. Therefore, this research focuses on the toxicity evaluation of toxic, acute and chronic organic loads on phenol degradation by a hybrid growth sequencing batch reactor.

1. Materials and Methods

1.1. HG-SBR setup and operation

The experiments were operated using a 7 L hybrid growth sequencing batch reactor (HG-SBR). The reactor was filled with 3 L of activated sludge from Shorubber (Malaysia) Sdn. Bhd. The biofilms grown onto the granular activated carbon (GAC) and bio-ring were added to the reactor as the material for the attached growth of microorganisms. The initial biomass concentration represented as mixed liquor suspended solids (MLSS) in the HG-SBR was 8300 mg/L.

The HG-SBR was operated with a cycle time of 24 hr per cycle. The advantage of the SBR system is that all operation modes can occur in the same reactor. The operation modes, consisting of Fill, React, Settle, and Draw, were assigned different time ratios. The time ratio for each mode was determined based on a previous study considering several factors such as the quantity of influent flow to be treated, strength of influent, number of parallel reactors, dissolved oxygen concentration, physicochemical stability of the activated sludge and the treatment requirement (Yusoff et al., 2016). The HG-SBR operated was in the time ratio of 1:20:2:1 for Fill:React:Settle:Draw, respectively.

During the Fill mode, 4 L of synthetic wastewater consisting of sucrose (563 mg/L), NH_4Cl (172 mg/L), $FeCl_3$ (12 mg/L), K_2HPO_4 (180 mg/L), KH_2PO_4 (35 mg/L) and $NaHCO_3$ (100 mg/L) were fed into the reactor. The pH of the synthetic wastewater was maintained at pH 6.8. The reactor was operated at room temperature. A submerged air diffuser was applied to provide aeration during the Fill and React modes. The aeration flow rate was maintained at 60 mL/min. A peristaltic pump (ProMinent: CONC 0308PP100AA02) was used to feed the influent from the influent tank to the HG-SBR.

1.2. Toxic organic loading (TOL)

Phenol solutions with the concentration of 50 mg/L were introduced into the HG-SBR system by mixing with the

synthetic wastewater. The HG-SBR was initially acclimatized with sucrose as the sole carbon source. The toxic effects were monitored for a period of 7 hr with sampling every 30 min. Samples collected were tested for chemical oxygen demand (COD) and phenol degradation. Dissolved oxygen was also observed during the 7 hr.

1.3. Acute organic loading (AOL)

The acute toxicity effect of the HG-SBR system was monitored with the increase of the phenol concentration. In order to evaluate the acute effect, monitoring was carried out separately after each new concentration was introduced. Five different concentrations were tested: 200 mg/L, 400 mg/L, 600 mg/L 800 mg/L and 1000 mg/L. During the first 7 hr of each new concentration, COD and phenol degradation profile tests were carried out in order to identify the acute effect towards HG-SBR as a function of increasing phenol concentration.

1.4. Chronic organic loading (COL)

Chronic organic loading was generated by differences in the phenol concentration introduced to the reactor. Different phenol concentrations were applied at different time periods for certain durations for observation of the chronic effects. Organic loading was increased up to 10-fold the normal concentration (100 mg/L). The COL was applied to the HG-SBR system for four days for each concentration. The HG-SBR then underwent recovery under the normal concentration of phenol.

1.5. Analysis method

Each of the samples obtained from the HG-SBR was analyzed for COD, phenol concentration, and mixed liquor suspended solids (MLSS). The COD was determined by using Method 5220C (APHA, 1992). Phenol concentrations were determined by a UV–Vis spectrophotometer (Hitachi 4-2800, Japan) from 200 to 800 nm. The maximum absorbance wavelength (λ_{max}) of phenol was 270 nm. The concentrations of MLSS were determined by following Standard Methods (APHA, 1992).

2. Results and discussion

2.1. Toxic organic loading (TOL)

The first stage of HG-SBR evaluation regarding toxicity was through assessment of toxic organic effects. Phenol with 50 mg/L concentration was introduced to the virgin activated sludge in the HG-SBR. The results obtained from TOL are presented in Fig. 1. It is apparent that the phenol concentration was reduced by 61% within 30 min of introduction. The result was comparable to the COD value, which exhibited 52% removal of the initial concentration. The decreases in the phenol and COD values within this duration were due to the adsorption of phenol to the surface of the activated sludge. Phenol was initially adsorbed by the activated sludge and then transported into the cell mass for the metabolic activities of cell growth (Tan and Chua, 1997). Adsorption on activated

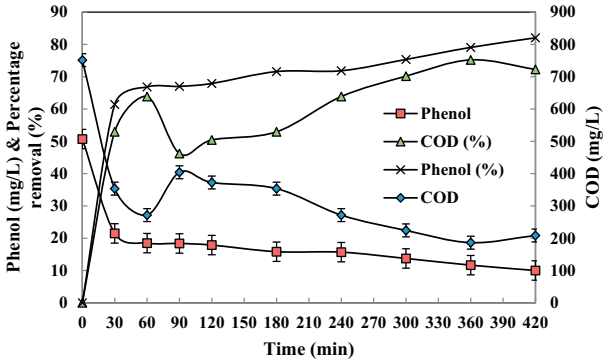


Fig. 1 – Phenol and chemical oxidation demand (COD) degradation for toxic organic loading (TOL). Phenol concentration 50 mg/L, Initial COD 751 mg/L.

sludge biomass is a crucial step for the biodegradation process (Seyhi et al., 2011). Some previous researchers have also reported a drastic reduction in the COD of wastewater at the instant the wastewater was mixed with activated sludge (Tan, 1993; Ting et al., 1994). The adsorption capacity obtained during TOL was 95.25 mg-COD/mg-MLSS for activated sludge, which expressed in terms of bio-sorption and calculated according to Eq. (2):

$$\begin{aligned} \text{Biosorption} &= (\text{COD}_{t=0} - \text{COD}_t)/\text{MLSS} \\ \text{Biosorption} &= ((751.29\ \text{mg COD/L}) \\ &\quad -(208.341\ \text{mg COD/L}))/(8.3\ \text{g MLSS/L}) \\ \text{Biosorption} &= 65.42\ \text{mg COD/g MLSS} \end{aligned} \tag{2}$$

The degradation continued to occur up to 60 min of reaction, but at a slower rate. The COD concentration increased slightly from 60 to 120 min of reaction. This observation could possibly be due to the formation of intermediates after the rapid metabolism of phenol by microorganisms. One of the most well-known dihydroxy aromatic intermediates resulting from the biodegradation of aromatic compounds is catechol (Loh and Chua, 2002; Seo et al., 2009). Catechol ($C_6H_6O_2$) has a similar structure to its parent compound phenol (C_6H_6O) and a maximum absorbance wavelength (λ_{max}) near that of phenol, at 280 nm (Lu et al., 2015). There is the possibility for bands of phenol and catechol to overlap during the UV–Vis spectrophotometer analysis, resulting in an increase in the concentration detected. In addition, catechol also contributes to the organic compound oxidization during the COD analysis.

Despite the apparent rapid degradation during the early period, the final percentage removals of phenol and COD were only 82% and 72% respectively. Co-metabolism could be a major contributing factor to this result. The co-metabolism process has been described as the degradation of a compound only in the presence of another organic material that serves as a primary growth substrate (Annadurai et al., 2008). Phenol was mixed with sucrose as a carbon source, for the HG-SBR, affecting the efficiency of microbial degradation. Co-metabolism contributed to the production of various specific enzymes, resulting in both phenol and sucrose competing for the same enzymes. The presence of metabolic inhibitors or competing substrates has a

significant impact on the biodegradation system. This was supported by Gladyshev et al. (1998), who reported that an increase in organic nutrients will decrease the biodegradation of phenol.

Fig. 2 provides the inter-correlation between the microorganisms' activities and dissolved oxygen. Microorganisms consumed oxygen during metabolic activities, and utilized dissolved oxygen as they consumed the organic compounds. The level of microorganism activities was indicated by the uptake of oxygen. The reduction of dissolved oxygen indicated to high oxygen uptake rates, which also indicated high levels of microorganism activity due to high amounts of organic compounds present, and vice versa. The dissolved oxygen dropped sharply from 6.91 to 1.52 mg/L. This was probably because the DO consumption was faster than the rate of oxygen supply (Mineta et al., 2011). Then, the dissolved oxygen gradually increased to 8.83 mg/L after 15 min. The dissolved oxygen continued to decrease up to 7 hr, indicating that continuous biodegradation of phenol occurred. Overall, the DO concentration profile was controlled by the balance between the oxygen consumption rate during biodegradation and the oxygen supply rate (Mineta et al., 2011).

2.2. Acute organic loading (AOL)

Acute is defined as sudden, severe or intense. AOL can be described as a sudden increase in the organic compound concentration. A sudden increment could possibly result in effects on the microorganisms and their cell activity. The concentration of phenol and the history of microorganism exposure to phenol are factors determining the alterations of the microorganism activity. The HG-SBR underwent increases in organic concentration during a certain period. The AOL effects were evaluated during the first cycle for each concentration. Fig. 3 shows the COD concentration profiles over 7 hr.

After the acclimatization period, 200 mg/L of phenol was introduced into the HG-SBR system. The COD concentration was gradually reduced from the initial value of 470 to 50 mg/L within 7 hr. The total percentage removal was 89.4%. Based on the COD degradation trend, there were no significant toxicity effects shown with 200 mg/L of phenol. Further analysis was continued

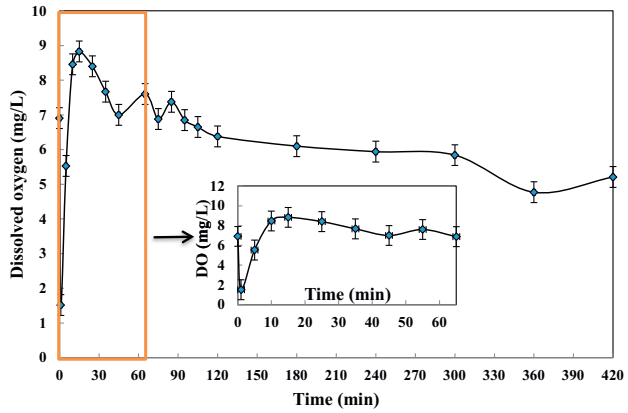


Fig. 2 – Dissolved oxygen profile during TOL. Phenol concentration 50 mg/L, initial COD 751 mg/L.

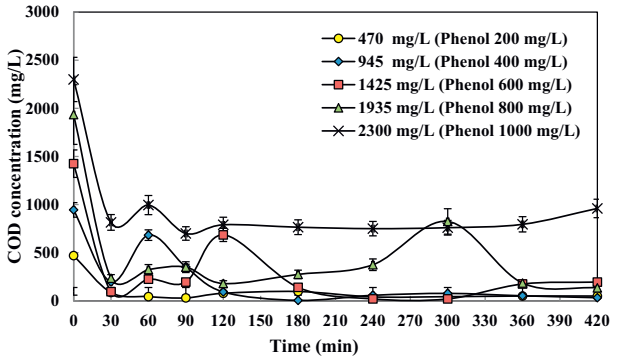


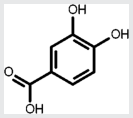
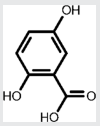
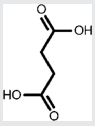
Fig. 3 – COD biodegradation profile for acute organic loading (AOL).

with phenol concentrations of 400, 600, 800, and 1000 mg/L. Before a new concentration was introduced, the HG-SBR system once again underwent recovery periods of 7 days with a normal phenol concentration (100 mg/L). It was observed that the toxicity effect became more significant as the concentration increased. The COD concentration significantly declined during the first 30 min of reaction. The initial COD decreased 79%, from 945 to 197.5 mg/L, for 400 mg/L of phenol. Higher degradation (93.3%) was recorded for 600 mg/L of phenol with the initial COD of 1425 mg/L, while 87.9% and 64.6% COD percentage removal was recorded for 800 mg/L and 1000 mg/L of phenol respectively.

An increase in COD could be observed for 60 min at all four of the higher phenol concentrations. When microorganisms are subjected to increased concentrations of toxic substances, their activity, as measured by the substrate degradation rate, diminishes until it reaches a point when all activity ceases (Comeau, 2008). Furthermore, the microorganisms have adaptive capabilities. The microorganism cells are capable of developing their enzymatic machinery to a level where the toxic organic compound can be used as a substrate (Comeau, 2008). Due to this adaptation, the microorganisms are capable of tolerating a high concentration of the toxic compound. This condition can be best illustrated by the higher removal observed for 600 and 800 mg/L compared to the removal for 400 mg/L of phenol. The microorganisms still in adaptation might progress when 400 mg/L is introduced to the HG-SBR system. Thus, the percentage removal was lower, but the COD level gradually decreased without any increment until 32.5 mg/L was reached at 7 hr.

In contrast, the COD concentration began to increase at 120 min for 600 mg/L and at 300 min for 800 mg/L phenol loading. This inconsistency of COD removal may be due to the formation of intermediates. The biodegradation of aromatic compounds such as phenol will initially produce intermediate compounds such as catechol (Khleifat, 2006; Stoilova et al., 2006; Jiang et al., 2007) protocatechuic acid, and gentisic acid (Loh and Chua, 2002). These intermediates further undergo ring fission following the Krebs cycle to yield other metabolic byproducts such as pyruvic acid, acetic acid, succinic acid and acetyl-Coenzyme A (acetyl-CoA) (Loh and Chua, 2002). The molecular structures of the intermediates are shown in Table 1. The formation of intermediates varies depending on the initial concentration of the parent compound. A high initial

Table 1 – Phenol biodegradation intermediates.

Intermediates compounds	Molecular structures
Catechol	
Protocatechuic acid	
Gentisic acid	
Pyruvic acid	
Acetic acid	
Succinic acid	

concentration will produce more intermediates, and may require a longer period for the reaction to fully occur.

Similar trends were observed for the degradation of phenol concentration, as shown in Fig. 4. The percentage of degradation tended to decline after two batches of phenol concentration were introduced. For instance, the percentage removal for 200 mg/L of phenol was 92.5%, and the removal increased to 97.3% for the introduction of 400 mg/L. The removal declined to 90.9% when the system was fed with 600 mg/L. However, the percentage removal increased back to 97.2% during feeding of 800 mg/L of phenol. This result indicated that as the toxic compound was continuously added, there was a possibility to halt the growth of heterotrophic microorganisms. The increase of the phenol concentration, which also acts as an inhibitory substrate, produces inhibition of the microbes. This can be reflected by the low removal efficiency of phenol observed at a certain concentration. While the increase of removal efficiency may be due to the completion of microorganism recovery and adaptation to the new phenol concentration, the recovery and adaptation tend to deteriorate at a certain concentration. However, phenol removal was able to achieve 90% efficiency at up to 800 mg/L, but not when 1000 mg/L of phenol was introduced into the system, which resulted in only 67% efficiency. This indicated that the optimum concentration that the system can withstand was around 800 mg/L.

The AOL effect was further evaluated based on the specific oxygen uptake rate (SOUR). Basically, SOUR is a conversion of the oxygen uptake rate value, giving a more accurate measure as it based on the biomass concentration. SOUR indicates the microbial activity based on the oxygen uptake rates and organic compounds present. A high oxygen uptake rate reflected high microbial activity due to high consumption of organic compounds. However, as shown in Fig. 5, higher phenol concentrations caused the decrease of SOUR for the microorganisms. The addition of phenol significantly affected the metabolic activity of microorganisms due to its toxicity. The reduction of SOUR increased by 27% when the concentration changed from 200 mg/L to 400 mg/L. SOUR gradually decreased as the phenol concentration increased. At the highest concentration of 1000 mg/L, a 62% SOUR reduction was recorded. The SOUR value decreased to 4.9 mg O_2/(g MLVSS·hr) at 1000 mg/L compared to 12.74 mg O_2/(g MLVSS·hr) at 200 mg/L. The reduction of SOUR values not only indicates low microbial activity, but is also due to the toxicity effects because the organic compound was phenol. At high concentrations, phenol inhibited microbial activity. The toxicity effects also caused a decreased in the biomass concentration. Thus, this consequently leads to a decrease in the SOUR value. The SOUR value recorded for 400, 600 and 1000 mg/L phenol was 9.29, 7.14, and 5.99 mg O_2/(g MLVSS·hr), respectively.

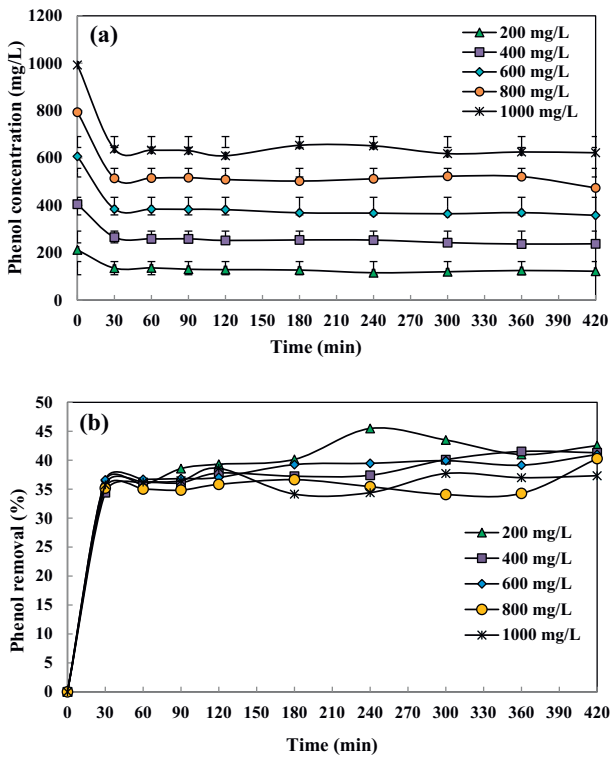


Fig. 4 – Phenol biodegradation profile for AOL.

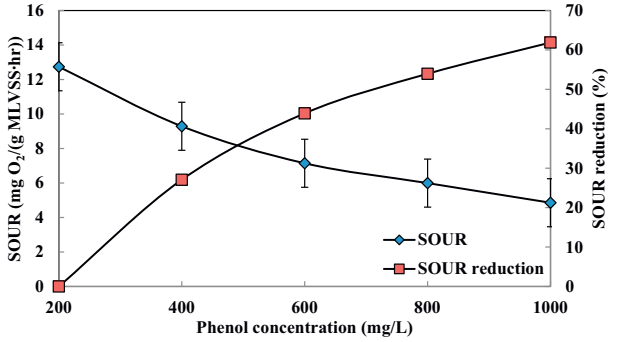


Fig. 5 – Specific oxygen uptake rate (SOUR) profile during AOL.

The trend of SOUR values at different concentrations, clearly indicated the importance of the presence of oxygen in determining the biodegradation of phenol. In the aerobic biodegradation of phenol, the initial step involved was oxygenation. The aromatic ring was monohydroxylated by a mono oxygenase phenol hydroxylase at a position ortho to the pre-existing hydroxyl group to form catechol (Sridevi et al., 2012). Catechol was the main intermediate produced during the metabolism of phenol by different types of microorganism strains. The subsequent intermediates formed depend on the types of microorganism strains. For instance, catechol undergoes ring cleavage at the ortho position, leading to the formation of acetyl Co-A. Different types of strains will lead to ring cleavage at the meta position, initiating the meta pathway and causing the formation of pyruvate and acetaldehyde. This clearly indicates that the utilization of oxygen molecules during biodegradation was crucial. More oxygen molecules were required as the phenol concentration increased to allow the aerobic biodegradation of phenol to occur.

2.3. Chronic organic loading (COL)

The HG-SBR system was monitored for 67 days under the stress of various phenol concentrations. The biodegradation of phenol through microorganism metabolism and other reactions contributed to the accumulation of living and non-living sludge or mixed liquor suspended solids (MLSS). The living parts in MLSS represented an adequate number of bacteria known as mixed liquor volatile suspended solids (MLVSS). Fig. 6 depicts the change of MLSS and MLVSS in the HG-SBR system.

The initial MLSS and MLVSS values were 6233 mg/L and 6000 mg/L respectively. Substrate uptake by microorganisms was used either for cellular growth or energy production. During cellular growth, small molecules accumulate to form large molecules. This contributed to an increase in sludge production; this condition is called anabolism (Gerardi, 2006). Anabolism usually occurs during the lag and log phases of microorganism growth. During the lag phase, microorganisms

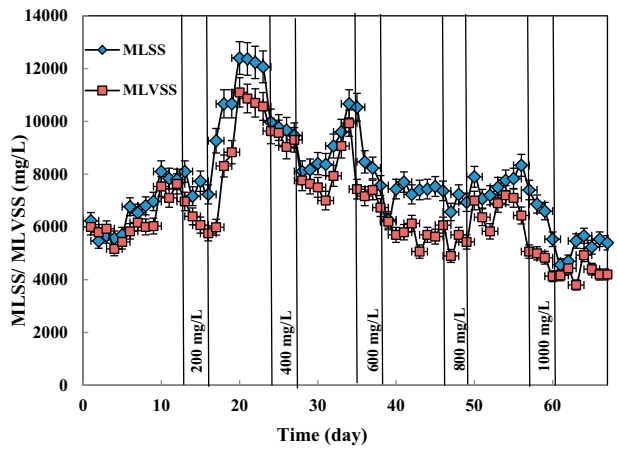


Fig. 6 – Mixed liquor suspended solids (MLSS) and Mixed liquor volatile suspended solids (MLVSS) during chronic organic loading (COL).

are active but do not reproduce. Microorganisms adjust to the new environment by synthesizing enzymes for the degradation of phenol (Gerardi, 2006). The duration of the lag phase depends on the conditions of the new environment and the species of the microorganisms. The lag phase could be observed, as the MLSS in HG-SBR slightly decreased to 5700 mg/L and MLVSS to 5833 mg/L after 12 days of acclimatization. The microorganism growth proceeded to the log phase, where the microorganisms continue to reproduce. Consequently, enzymes for the degradation of phenol kept being produced by the microorganisms, related to the increased growth of microorganisms. New microorganism cells continued to be synthesized, and the growth of microorganisms was most rapid during this log phase. This can be proven by the increase of the MLSS and MLVSS values to 7800 mg/L and 7633 mg/L respectively. However, the MLSS value for 200 mg/L of phenol addition fluctuated a little. A sudden increase occurred during the third day loading. As 2-fold load was introduced to the system, the MLVSS value gradually decreased and dropped from 6967 to 5760 mg/L within 4 days. The growth rates of microorganisms were slightly slower due to the sudden increase of toxicity. After this high load, the HG-SBR system went through a recovery period, as both the MLSS and MLVSS value increased during this period of time. The system recovered immediately and continued to show increments for both values. This condition indicated that the microorganism growth returned to the lag phase. This was normal, as the lag phase usually occurs during the start-up of the biological treatment or during the recovery from toxicity (Gerardi, 2006). This also could possibly explain why the effects of chronic organic loading were not severe during the 200 mg/L organic loads.

Subsequently, during the 400 mg/L loading, both MLSS and MLVSS values decreased to 9967 mg/L and 9633 mg/L respectively. MLVSS continued to decline to reach 9300 mg/L. There was some decrement recorded for the MLSS value. MLSS and MLVSS continued to decrease during the recovery period, but slowly increased after a few days. Similar decreasing patterns were observed when 600 mg/L of phenol was introduced to the reactor. The final MLSS and MLVSS were measured after 4 days of exposure to 600 mg/L of phenol and recorded at 7567 mg/L and 6733 mg/L respectively.

After certain periods, the microorganism growth tended more toward catabolism. Most of the substrate was used for energy production. Large molecules of the substrate were degraded to smaller molecules, and cellular energy was obtained (Gerardi, 2006). This also contributed to the decrease of sludge production. Catabolism occurred with the onset of the endogenous phase of growth (Gerardi, 2006). The microorganisms had most probably reached their carrying capacity. After the 600 mg/L loading, both MLSS and MLVSS values were in equilibrium until recovery after 800 mg/L of phenol was added. Basically, there was no obvious increase in microorganism growth. Most of the cellular growth was balanced by cellular death during the endogenous phase of growth. The microorganisms could not grow further with increasing quantities of the substrates. The production and accumulation of toxic substrates also contributed to this condition. However, there was an obvious decrease in the MLSS and MLVSS values during the 1000 mg/L phenol loading, which was probably due to the

microorganisms reaching the death or decline phase. During this phase, the death rate exceeds the growth rate of microorganisms. Therefore, it seems that time was a crucial factor when dealing with exposure. The adaptation of microorganisms was influenced both by short-term or long-term exposures. A low loading concentration at the early stage provides better adaptation and consequently reduces the chronic effects.

As shown in Fig. 7, the COD removal efficiency was different for each concentration. The removal efficiency during 200 mg/L loading was 82%–85% throughout 4 days. The COD removal efficiency decreased to 72%–76% during 400 mg/L loading with initial COD of 1108 mg/L. The same pattern was recorded for phenol removal, as shown in Fig. 8; the percentage removal of phenol ranged between 83%–85% and 72%–76% at 200 mg/L and 400 mg/L loadings respectively. Both the COD and phenol removal efficiencies continued to decrease as the organic loading increased. With the initial COD value of 2062 mg/L, the removal efficiency was within the range 64%–71%. Phenol removal was able to reach 51%–76% at 600 mg/L loading. The effect of COL was more apparent at 800 mg/L and 1000 mg/L phenol concentration with the initial COD values of 2538 mg/L and 3015 mg/L respectively. For COD, the removal range was between 22%–30% for 800 mg/L loading. Further decrement of phenol was recorded within the range of 30%–44%. Lower degradation efficiency was recorded at 1000 mg/L loading. Only

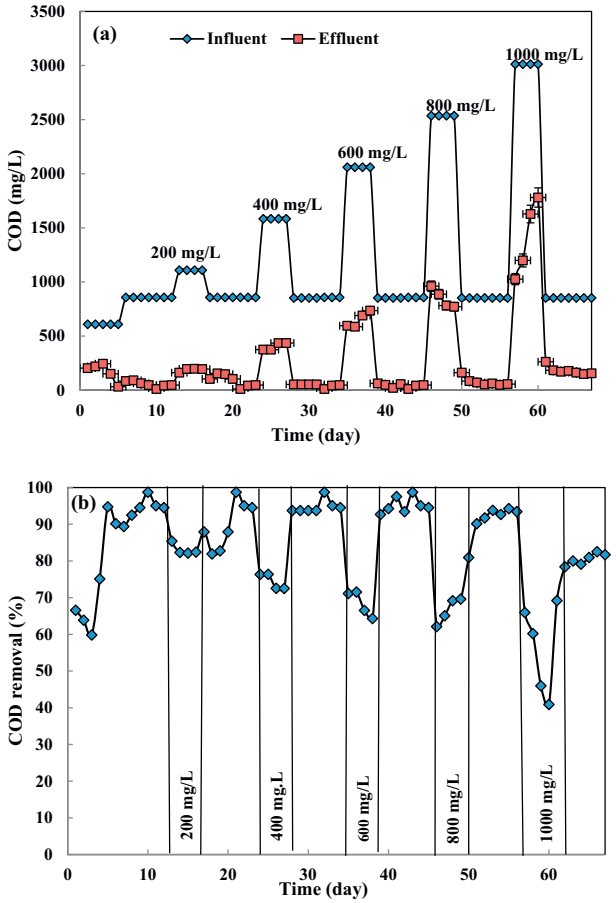


Fig. 7 – COD removal efficiency for chronic organic loading (COL).

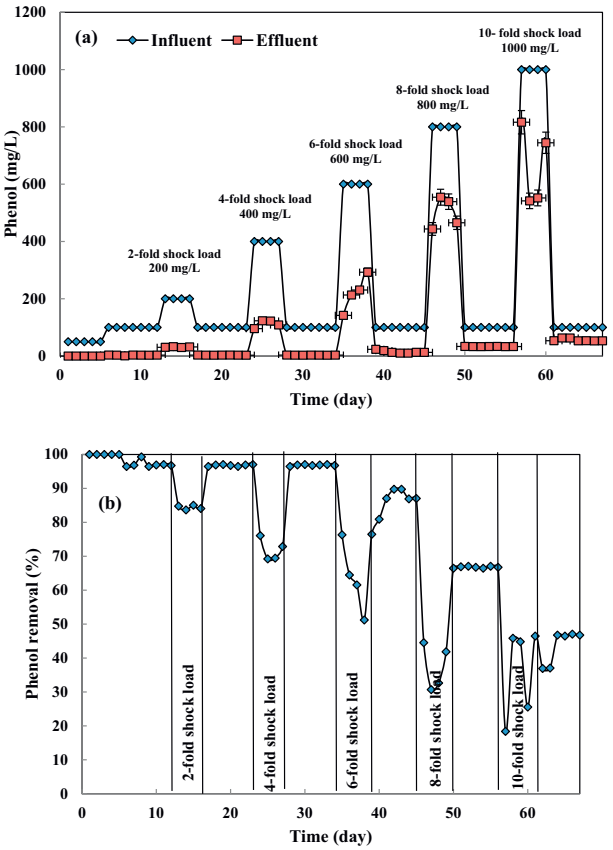


Fig. 8 – Phenol removal efficiency during COL.

7%–14% of COD removal and 18%–46% phenol degradation was observed.

3. Conclusions

The performance of the hybrid growth sequencing batch reactor (HG-SBR) was different for the three conditions of toxicity studied (toxic, acute and chronic). In the case of TOL, the system was adversely affected with the sudden introduction of phenol to virgin activated sludge. Phenol and COD were reduced by 61% and 52% respectively within 30 min of reaction. The decrease of phenol concentration and COD value within this duration was due to the adsorption of phenol to the surface of the activated sludge before being transported to the cell mass, with the adsorption capacity of 65.42 mg-COD/g-MLSS. However, the formation of intermediates and co-metabolism could be contributing factors to the low degradation rate after 30 min of reaction. The dissolved oxygen dropped sharply from 6.91 to 1.52 mg/L, indicating the high dissolved oxygen consumption during TOL. AOL showed significant effects at high phenol concentrations. The COD value declined 79% for 945 mg/L initial concentration. Higher degradation (93.3%) was recorded for 1425 mg/L initial COD, while, 87.9% and 64.6% of COD removal was recorded for 1935 mg/L and 2300 mg/L initial COD respectively. The microorganism activities, which were measured by the substrate degradation rate, diminished until it reaching a point where all activities ceased. SOUR gradually decreased as

the phenol concentration increased, indicating that the toxicity affected the amount of oxygen consumed for microbial activity. The HG-SBR was further monitored for COL over 67 days. The trends for biomass concentration as represented by MLSS and MLVSS were similar to the theoretical microbial growth curve. There was no obvious increase of biomass concentration during the lag phase, where microorganisms are active but do not reproduce. There was an increase in the toxicity effect on the growth of microorganisms, but they were able to recover during the recovery period. However, after loading with 600 mg/L phenol, the microorganisms could not grow further. Overall, the phenol and COD removal decreased as the loading concentration increased from 200 mg/L to 1000 mg/L, indicating the severe effects of COL.

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