# Biodegradation of dihydroxybenzenes (hydroquinone, catechol and resorcinol) by granules enriched with phenol in an aerobic granular sequencing batch reactor

A Thesis

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# ABSTRACT

During the last 20 years, intensive research in the field of wastewater treatment has been conducted to focus on aerobic granular sludge because of its efficiency for water purification. Previous researchers have studied the characterization of the granules and the granular sequencing batch reactor's (GSBR) applications. However, there is little research done on how enrichment with one compound influences the biodegradation of structurally similar compounds. In this research, phenol-enrichment was investigated as a potential method to improve the biodegradation of the three dihydroxybenzenes (hydroquinone, catechol and resorcinol), which are structurally similar to phenol and even share biodegradation pathway reactions. The objectives of this research were: (1) Cultivate stable aerobic phenol-degrading granular sludge using acetate-fed granules as microbial seed; (2) Compare the removal over one anaerobic/aerobic cycle of the three dihydroxybenzene compounds (hydroquinone, catechol and resorcinol) added individually by the phenol-enriched acetate-fed granules (PAFG) reactor and the non-phenol-enriched acetate-fed granules (NPAFG) reactor (control); (3) Compare the removal of phenol and the three dihydroxybenzene compounds (hydroquinone, catechol and resorcinol) added individually during the anaerobic phases and the aerobic phases; (4) Compare the removal of the dihydroxybenzenes when they are provided as a mixture to the PAFG and the NPAFG reactors; (5) Discuss implications for GSBR operation to treat aromatic chemicals. Two reactors, the PAFG reactor and the NPAFG (control) reactor, were created and used in this project. GSBRs are thought to require both anaerobic and aerobic phases (and phosphorous-accumulating organisms) in order to form granules and there are anaerobic pathways for degradation of phenolics, so to determine the possible significance of the anaerobic degradation one GSBR cycle included both anaerobic and aerobic phases in this study. Stable aerobic

acetate-degrading granules were cultivated first using acetate-containing synthetic wastewater of 600 mg COD/L (chemical oxygen demand), resulting in two identical reactors, both with more than 90% acetate-removal and a 6-hour hydraulic retention time (HRT). Then the PAFG reactor was fed synthetic wastewater containing acetate and phenol at a total of 600 mg COD/L, while the NPAFG reactor was still fed with acetate-containing synthetic wastewater at 600 mg COD/L. In the PAFG reactor, phenol concentrations were gradually increased from 10 mg/L to 100 mg/L (246 mg COD/L). Removal tests of three dihydroxybenzenes were conducted individually at 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L and the removal test of a mixture of the four compounds stated above was conducted at 50 mg/L each. Samples from degradation experiments with phenol and dihydroxybenzenes were analyzed and quantified with high-performance liquid chromatography using a UV detector (HPLC-UV) to analyze phenol and the three dihydroxybenzenes and NanoDrop to analyze COD concentrations via colorimetric COD assays. After phenol-enrichment, in the PAFG reactor phenol removal percentages were more than 90% in a single cycle. Overall, of the three dihydroxybenzenes tested, hydroquinone was removed most effectively (80% – 90% in a single cycle) in both the PAFG and the NPAFG reactors at all the four concentrations. There was 20% increase of the catechol removal in the PAFG reactor (80% removal on average) compared with the NPAFG reactor (60% removal on average). Resorcinol removal percentages were lowest overall but they showed the greatest improvement after phenol-enrichment. In the NPAFG reactor, only 20% of resorcinol was removed during one GSBR cycle, while in the PAFG reactor, around 60% of resorcinol was removed. During one cycle, most of the aromatic compounds were degraded in the aerobic phase (most rapidly during the first 40 minutes of the aerobic phase). The observed aerobic degradations of phenol, hydroquinone and catechol were consistent with several kinetics models (first-order, Monod or

Haldane kinetics) and the best model cannot be chosen without follow-up studies with many more data points and a wider range of concentrations tested. Together, data from these experiments demonstrated that phenol-enrichment of GSBR granular sludge can improve the biodegradation of the three dihydroxybenzenes (hydroquinone, catechol and resorcinol), which can be potentially used in wastewater treatment.

# BIOGRAPHICAL SKETCH

Jing Zhao was born in Shijiazhuang, China in 1991. She graduated with a degree in Environmental Science from Minzu University of China in 2014. At her third year in college, she went to University of California, San Diego for one-year exchange. From Dec. 2012 to Aug. 2013, she worked as an undergraduate research assistant in Scripps Institution of Oceanography, UC San Diego focusing on algae technology. During her undergraduate, she got National Scholarship twice and Outstanding Graduate of Beijing.

The attractive academic atmosphere in the United States prompted her to pursing mater degree there. In August 2014, she entered Cornell University and started her study towards the M.S. Degree in Environmental Engineering.

# ACKNOWLEDGEMENT

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Last but not least, I would like to thank Dr. Damian Helbling for generously letting me use the high-performance liquid chromatography (HPLC) in his lab. I would not successfully finish my thesis without it. And thanks Corey Carpenter for teaching me how to use the HPLC.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **COD** | chemical oxygen demand |
| **DO** | dissolved oxygen |
| **GSBR** | granular sequencing batch reactor |
| **HPLC** | high-performance liquid chromatography |
| **HRT** | hydraulic retention time |
| **NPAFG** | non-phenol-enriched acetate-fed granules |
| **PAFG** | phenol-enriched acetate-fed granules |
| **SBR** | sequencing batch reactor |
| **SVI** | sludge volume index |
| **TSS** | total suspended solids |
| **VSS** | volatile suspended solids |

# CHAPTER 1: INTRODUCTION

## *1.1 Context*

During the last 20 years, intensive research has been conducted in the field of granular biomass technology. It has been demonstrated that aerobic granules were often more efficient for wastewater purification than suspended activated sludge.[1] To date, the application of aerobic granular sludge is regarded as one of the promising biotechnologies in wastewater treatment.

Before the studies of aerobic granules, granulation was used only in anaerobic sludge. In 1980, granular sludge was shown to be effective for wastewater treatment in upflow anaerobic sludge blanket (UASB) reactors.[2] Wirtz and Dague (1996) hypothesized that granulation would be encouraged by a short settling time causing poor-settling biomass to wash out, and a high organic loading rate, ensuring sufficient new biomass growth.[3] By 1996, granulation was only known to occur in anaerobic sequencing batch reactors[3] and in biofilm airlift suspension reactors[4]. Granules have been defined as compact, spherical, self-attached biofilms that are suspended during mixing.[5] Granules showed much better settling properties and an ability to withstand high loading rates even when densities of granules were equal to densities of discrete bacterial cells or loosely packed bioflocs.[6]

The anaerobic granulation technology exhibited several drawbacks that included a long start-up period, a relatively high operating temperature, unsuitability for low-strength organic

wastewater, and low efficiency in the removal of nutrients (N and P) and pathogens from wastewater. These drawbacks encouraged the development of aerobic granular technology.[1]

By the late 1990s, the formation and application of aerobic granules have been reported.[7–9] The first patent was granted by Heijnen and van Loosdrecht.[10] De Kreuk et al. provided a kinetic model for the aerobic granulation process.[11,12] Liu and Tay (2004) and Maximova and Dahl (2006) provided a comprehensive summary of current understanding towards the bioaggregation processes.[13,14]

Compact structured aerobic granules with diverse microbial species and excellent settling capabilities have been cultivated in sequencing batch reactors (SBR).[7,8,1516] These SBRs were cycled through phases of: feeding, optional anaerobic period, bubbler-based aeration, settling and discharge. Aerobic granule formation mechanisms have been studied by several researchers.[17–22] The aerobic granules were reported to exhibit attributes of:[1]

1) Excellent settleability
2) Dense and compact microbial structure
3) High biomass retention
4) Ability to withstand high organic loading
5) High tolerance to toxic compounds

Because of the unique granule characteristics, the aerobic granulation technology was recently developed for treating various high strength wastewaters containing organics, nitrogenous compounds, phosphorus, toxic substances and xenobiotics.[1,16,23–25]

## *1.2 Objectives*

Experiments described in this thesis were designed to expand the possible applications of GSBR (granular sequencing batch reactor) to the treatment of mixtures of aromatic chemicals. Phenol and the three dihydroxybenzene isomers (hydroquinone, catechol and resorcinol) that were selected are simple aromatic compounds and are common in industrial processing and consumer products. Four main objectives were as follows:

1) Cultivate stable aerobic phenol-degrading granular sludge using acetate-fed granules as microbial seed.
2) Compare the removal over one anaerobic/aerobic cycle of the three dihydroxybenzene compounds (hydroquinone, catechol and resorcinol) added individually by the phenol-enriched acetate-fed granules (PAFG) reactor and the non-phenol-enriched acetate-fed granules (NPAFG) reactor (control).
3) Compare the removal of phenol and the three dihydroxybenzene compounds (hydroquinone, catechol and resorcinol) added individually during the anaerobic phases and the aerobic phases.
4) Compare the removal of the dihydroxybenzenes when they are provided as a mixture to the PAFG and the NPAFG reactors.
5) Discuss implications for GSBR operation to treat aromatic chemicals.

## *1.3 Hypotheses*

The hypotheses are:

1) Phenol-degrading granular sludge could be cultivated rapidly using acetate-fed granules as microbial seed.
2) Phenol-enrichment could enrich organisms that could also degrade dihydroxybenzenes.

# CHAPTER 2: BACKGROUND

## *2.1 Granular sequencing batch reactor*

### 2.1.1 Granule discovery

Before the study of aerobic granules in sequencing batch reactors (SBR), granular sludge was already being effectively used in strictly anaerobic systems to treat domestic and industrial wastewaters.[2] The formation of aerobic granules has been reported by the late 1990s.[7] Aerobic granules inoculated using sludge from a municipal wastewater treatment plant were first successfully cultivated in a laboratory-scale SBR that cycled through anaerobic and aerobic phases with 500 mg COD/L COD (chemical oxygen demand) load in 1997. The SBR was operated with a very short sedimentation time (60 seconds) to enhance the growth of rapidly-settling and compact granular sludge.[7] Although after about 130 days of operation the granule quality and COD-removal worsened[7], the study successfully opened the door for research regarding aerobic granular biotechnology. By the end of 1990s, high COD removal (2.16 g TOC (total organic carbon) · g $SS^{-1}$ · d) and nitrification activities (0.24 g $NH_3$-N · g $SS^{-1}$ · d) were achieved in GSBRs that were inoculated using activated sludge from a municipal wastewater treatment plant. This was possible even under low dissolved oxygen (DO) (0.8 mg/L) with aerobic granular sludge (Sludge Volume Index (SVI) between 80 - 100 mL/g) inoculated using sludge from a municipal wastewater treatment plant. This study proved the aerobic granules have high degrading ability when treating wastewater, good settleability and efficiency under low DO concentrations.[9] Other researchers studied the basic requirements for granulation. The dominant requirement is a short settling time, allowing for the wash-out of flocculated sludge[7]. High COD

loading and low shear force lead to the outgrowth of filaments and hinder the formation of aerobic granules.[8] These initial studies led to follow-up studies on the granulation processes.

### 2.1.2 Granule formation

Granulation was affected by a number of operational parameters, such as: seed sludge or other inoculum, wastewater compositions and SBR operational parameters (pH, temperature, cycling time, and others).[18,26]

#### *2.1.2.1 Seed sludge*

In most studies, aerobic granules were cultivated with activated sludge seed.[7–9,27] The bacterial community residing in activated seed sludge was important for the aerobic granulation process. Bacterial cell surface hydrophobicity is one of the most important factors that influence bacterial adhesion[28] and directly influences the formation and structure of granules which are associated very closely with cell hydrophobicity. Cell hydrophobicity, a triggering force of granulation, is a prerequisite of granulation.[26] The aerobic granulation could be faster with more hydrophobic bacteria in the seed sludge.[29]

#### *2.1.2.2 SBR operation*

Settling time, cycle time, starvation phase time, temperature and hydrodynamic shear force[30] all impact the aerobic granulation.

Short settling time allowed the wash-out of flocculated sludge and retained only well settled granules.[7–9,16–18] Studies have indicated that short settling time could enhance aerobic granulation.[17]

Cycle time influences the biomass growth rate of granular sludge and the size of the granules. The specific biomass growth rate of granular sludge decreased from 0.266 to 0.031 $d^{-1}$ (the corresponding biomass growth yield decreased from 0.316 to 0.063 g VSS $g^{-1}$ COD), when cycle time increased from 1.5 to 8 h. And the granules cultivated at 1.5 h cycle time were the biggest in size, while the granules cultivated at 4 h cycle time were the most compact ones compared with those cultivated at other cycle times. Short cycle time could result in high organic loading rate and short hydraulic retention time. However, too short cycle time is not advisable because of more excessively produced sludge and higher observed specific biomass growth rate.[31]

The length of the COD-starvation time at the end of the aeration phase (after all COD conversion has occurred) has been reported to impact aerobic granulation and granule stability.[32,33,34] In acetate-fed GSBR studies, a long starvation period (7.3 h starvation period in an 8 h cycle) resulted in weak granules and poor reactor efficiency and a very short starvation period (0.8 h starvation period in a 1.5 h cycle) led to a mix of fluffy and compact granules after a four month operation period[34], which may lead to unstable performance. An intermediate starvation period (3.3 h starvation period in a 4.0 h cycle) led to the most compact granules and the highest overall density of biomass.[33]

Most aerobic granular sludge research was carried out at room temperature (20 - 25 °C).[7–9,16–18,26] Granules yielded at 8 °C were irregular in shape and with outgrowth of filamentous organisms causing severe biomass washout and unstable.[36] Aerobic granulation was not possible at low temperatures with the present understanding.

### 2.1.3 Aerobic GSBR applications

#### *2.1.3.1 Removal of COD, nitrogen and phosphorus*

The removals of COD, nitrogen and phosphorous were major foci of GSBR studies after the discovery of granules.

It has been demonstrated that nitrification (conversion of ammonium to nitrite and nitrate using oxygen as an electron acceptor), denitrification (reduction of nitrate to nitrite and ultimately nitrogen gas), and COD removal can occur simultaneously in a granular sludge SBR under specific cycle conditions.[16] The distribution of the nitrifier biomass is determined by the dissolved oxygen (DO), which influences the ammonium removal. The absence of oxygen and availability of $NO_2^-$ (nitrite), $NO_3^-$ (nitrate) and COD are required for denitrification. Absence of oxygen in the center of the granules could be achieved by decreasing the DO to a level just high enough to accomplish nitrification in the outer layer of granules.[16] Availability of COD for denitrification can be achieved by successive feeding of COD and then oxygen into the GSBR. Acetate was partly (40% on mole of carbon basis) stored internally as poly-ß-hydroxybutyrate (PHB). When acetate was depleted, PHB was used as a carbon source and energy source for

denitrification in deeper layers of the granules.[32] Complete phosphorus, nitrogen and COD removal have been achieved in a GSBR optimized for phosphorus removal via Enhanced Biological Phosphorus Removal (EBPR) (influent N: 20 mg/L, P: 20 mg/L, COD: 220 mg COD/L). EBPR biomass has shown a dominance of *Accumulibacter* spp. (a polyphosphate-accumulating organism, PAO) and *Competibacter* spp. (a glycogen non-polyphosphate-accumulating organism, GAO). *Accumulibacter* spp. was dominant in the outermost 200 μm region of the granule while *Competibacter* spp., responsible for denitrification, dominated in the granule central zone.[37]

Simultaneous nitrification and denitrification optimization in an aerobic granular sludge system was performed using different oxygen concentrations, showing that decreased oxygen concentration was required to obtain effective denitrification and low aeration energy requirement. Oxygen reduction (from 100% to 50%, 40%, 20% or 10% of the saturation concentration) by blending nitrogen and air in the gas-phase in one cycle did not influence the acetate uptake rate. Nitrogen removal (defined here as complete conversion to nitrogen gas) was improved with the decrease of the oxygen concentration, reaching a value of 34% at 10% of the oxygen saturation concentration.[38] Characterization and evaluation of the aerobic granules showed that ammonia oxidizing bacteria (AOB, which performed nitrification) existed primarily in the outer layers of the granules, with most of the nitrification likely occurring from the surface to 300 μm into the granular thickness, if a first-order reaction for nitrification was assumed.[39]

EBPR granules containing poly-phosphate-accumulating bacteria uptake soluble organic carbon and release phosphate in the anaerobic phase of the SBR cycle and during the aerobic phase they

rapidly uptake phosphate.[1] Phosphorus-accumulating microbial granules were developed at different substrate P/COD ratios in the range of 1/100 to 10/100 by weight in GSBRs with 500 mg COD/L influent levels. The accumulated phosphorus showed a decreased trend with the increase in substrate P/COD ratio. Influent with a P/COD ratio of 2.5% resulted in granules with a P content of nearly 6%.[25]

#### *2.1.3.2 Use of GSBRs to treat toxic organic wastewaters*

Because of the structure of the granules resulting in the protection of biomass inside granules, aerobic sludge granules have also been applied to treat toxic organic wastewaters such as industrial wastewaters.

Tay and coworkers rapidly cultivated stable aerobic phenol-degrading granules using acetate-fed granules as microbial seed and a cycle time of 4 hours (229 min aerobic phase). Aerobic granules were first cultivated in SBRs with acetate as sole carbon source at a loading rate of 3.8 kg $m^{-3}$ $d^{-1}$. After stable granules were formed, phenol replaced acetate in influent to the four reactors at loading rates from 0 to 2.4 g $L^{-1}$ $d^{-1}$. The granules stabilized within one week and complete phenol removal was achieved. The benefits of using aerobic granules cultivated on benign substrates as microbial seed to produce granules to degrade toxic substrates were first demonstrated.[40]

Other research demonstrated that step increases of the concentration of toxic chemicals could be used to achieve granule acclimation. Compact and well-settling aerobic granules were developed

in SBRs for the biological removal of tert-butyl alcohol achieved through increasing tert-butyl alcohol concentrations in the influent step by step.[41] Tert-butyl alcohol is directly added to fuels as an octane index enhancer to reduce vehicle emissions and is also widely used as a solvent in the manufacturing of plastics, resin polymers, perfumes, paint removers, insecticides, and pharmaceutical products.[42] Additionally it can be produced as a by-product of partial biodegradation of methyl-tert-butyl-ether (MTBE).

Aerobic granules were also successfully developed to treat wastewater containing p-nitrophenol, an industrial chemical, in a SBR using activated sludge as inoculum.[43] Moreover, biodegradation of 2-fluorophenol, m-cresol, pyridine and isopropyl alcohol by aerobic granular sludge in SBRs have all been successfully achieved at the laboratory scale.[44–47] Aerobic granular sludge technology shows great potential for biologically treating industrial wastewaters but must be proven in more situations and over long operating times.

### 2.1.4 GSBR technology in full scale

Although the study of granular sequencing batch reactors only started in the late 1990s, there are already full-scale wastewater treatment plants using aerobic granular sludge technology. Recently, aerobic granular sludge technology has been scaled-up to treat domestic wastewater under the trade name Nereda® (Royal HaskoningDHV). A robust and stable granule bed (> 8 g $L^{-1}$) with a sludge volume index after 5 min settling of 45 mL $g^{-1}$ was formed after a start-up period of approximately 5 months (dry weather conditions led to a 6.5-h cycle and rainy weather led to a 3 h cycle). The total COD load was 14,636 kg COD $day^{-1}$. More than 80% of granules

were larger than 0.2 mm and more than 60% were larger than 1mm. Effluent requirements (7 mg $N\ L^{-1}$, 1 mg $P\ L^{-1}$, 125 mg COD $L^{-1}$and 30 mg TSS $L^{-1}$) were met and maximum volumetric conversion rates for nitrogen and phosphorus were respectively 0.17 and 0.24 kg $(m^3\ d)^{-1}$. Moreover, the energy usage was 13.9 kWh $(PE_{150}\ year)^{-1}$ (P.E.[48]: Population equivalent; 1 P.E. equals 150 g Total Oxygen Demand per day) which was 58 - 63 % lower than the average conventional activated sludge treatment plant used to treat the same wastewater volume per day. This study demonstrated that aerobic granular sludge plant can effectively be implemented at full scale for the treatment of domestic wastewater and the reactor volume needed was 33% lower than that for the existing conventional activated sludge treatment plant.[48] However, the granular GSBR technology will need to operate stable for many years before it is proven as mainstream wastewater treatment technology.

## *2.2 Phenol, hydroquinone, catechol, resorcinol and their detailed biodegradation pathways*

### 2.2.1 Phenol toxicity and biodegradation

Phenol and its derivatives are one of the largest groups of environmental pollutants because of their broad applications (e.g., as antibacterial and antifungal agents), and their presence in many industrial productions. Pollution by phenolic compounds should be avoided because most of these compounds exhibit varying degrees of toxicity.[49] Skin irritation, gastrointestinal discomfort, and headaches can be caused by acute exposure to phenol. Besides, phenol is also toxic to the nervous system, the heart, the kidneys, and the liver.[50] The toxicity of phenol towards plants has been studied. When exposed to 1,000 mg/L phenol, willow trees wilted and died at the

end of 120 h.[50] Phenol can inhibit synthesis and replication of DNA in cells. A study revealed that phenol stopped preparation of DNA in diploid human fibroblasts.[51]

To treat wastewaters containing phenol, aerobic biodegradation of phenol is preferred because aerobic microorganisms can rapidly mineralize phenol to $CO_2$. Phenol is recognized as an inhibitory substrate at relatively low concentrations (100 mg/L)[52]. Because microbial growth is inhibited by higher concentrations of phenol, in order to treat high concentration phenol waste various approaches have been developed to overcome this. By increasing the phenol concentration step wise, microorganisms can be adapted to higher phenol concentrations. Biofilm formation has been beneficial to phenol wastewater treatment due to the biofilm's protection of deeper bacteria from toxicity.[53] Targeted metabolic engineering has been used in industrial strain improvement to overcome the inhibition by higher concentrations of phenol.[54] Paraskevi and Euripides have successfully isolated microorganisms from petroleum-contaminated soil in Denmark, which used phenol as a sole source of carbon and energy.[55] Kafilzadeh and coworkers (2010) isolated and identified phenol-degrading bacteria from Lake Parishan. Different concentrations of phenol (from 200 to 900 mg/L) were used to test the ability of bacteria to degrade phenol. Most of the isolated bacteria showed a good ability of phenol degradation even at high starting phenol concentration. When fed 900 mg/L phenol, *Pseudomonas* and *Acinetobacter* showed 800 - 900 mg/L of phenol degradation, and *Kelibsiella*, *Citrobacter* and *Shigella* showed 600 - 700 mg/L of phenol degradation.[56] Known aerobic pathways for the degradation of phenol are shown in Figure 1.

Phenol can also be degraded in the absence of oxygen (Figure 1). In this pathway, phenol is carboxylated in the para position to 4-hydroxybenzoate which is the first step in the anaerobic pathway. Here, the enzyme involved is the 4-hydroxy-benzoate decarboxylase.[57] The organisms are capable of degrading phenol under anaerobic conditions are *Thauera aromatica* and *Desulphobacterium phenolicum* [57]. Veeresh et al. used an upflow anaerobic sludge blanket (UASB) bioreactor for treatment of phenolic wastewater. Acclimatization of a wide variety of inocula such as anaerobic sludge from pulp-mill lagoon, municipal activated sludge adapted to phenol and sludge from anaerobic digester all generated phenol-degrading UASB communities. With acclimatization of the inocula, recirculation of the treated effluent and supplementing with co-substrates such as glucose, phenol concentrations greater than 500 mg/L were effectively treated with a 2.5 day HRT.[58] Azbar et al. used anaerobic hybrid reactor combining sludge blanket in the lower part and filter in the upper part to treat phenol-containing wastewater (1000 - 4000 mg/L) and achieved phenol removal efficiencies ranging from 39 to 80 % (0.45 - 32 kg COD $m^{-3}$ $day^{-1}$)[59].

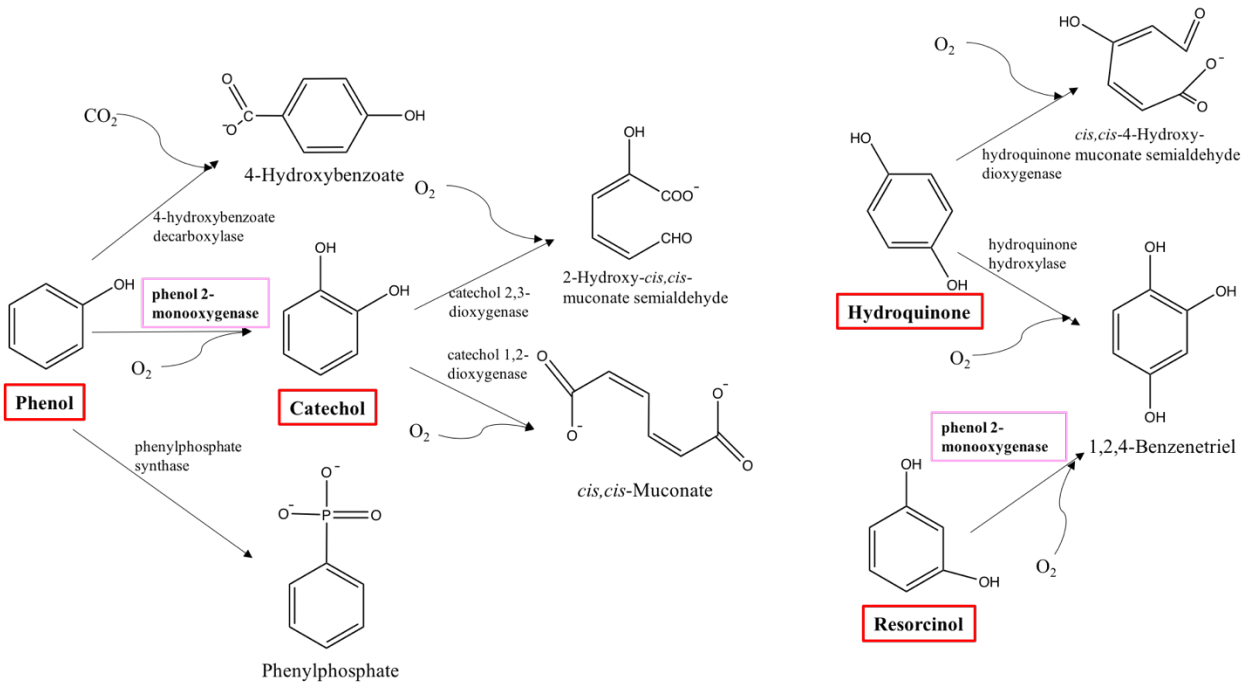



**Figure 1.** Initial steps of known biodegradation pathways for phenol, hydroquinone, catechol and resorcinol as presented at the EAWAG Biocatalysis and Biodegradation database (http://eawag-bbd.ethz.ch/search/). Intermediates lead into other common pathways.

## 2.2.2 Hydroquinone toxicity and biodegradation

Hydroquinone (benzene-1,4-diol), a major benzene metabolite, is a ubiquitous chemical in the environment due to its widespread application in consumer and industrial products. It is widely used as a developing agent in photography, dye intermediate, stabilizer in paints and varnishes oils, and motor fuels. In addition, hydroquinone has been used as an antioxidant in the rubber and food industry.[60]

Since the 1950s hydroquinone (HQ) has been used in skin lightener products and since the 1960s it has been used in medical products. It is also widely used in cosmetic products such as hair dyes and products for coating finger nails[61]. From 2001 onward, HQ was no longer authorized

for use in cosmetic skin lightening formulations in European Union countries.[61] Additionally, hydroquinone can be a component of high-molecular-weight aromatic compounds (e.g., resin), or appear as an intermediate generated by transformation of aromatic compounds. Dihydroxybenzenes (hydroquinone, catechol and resorcinol) can be formed during the advanced oxidation processes of aromatic compounds (e.g., phenol) as intermediate oxidation products.[62]

Biodegradation using microbial biomass is an effective strategy for removing hydroquinone. Hydroquinone can be degraded by two different pathways depending on the oxygen availability (Figures 1, 2 and 3). Under aerobic conditions hydroquinone is transformed through two different metabolic pathways to metabolites that enter the beta-ketoadipate pathway (Figure 2)[63,64]. The first pathway involves the initial hydroxylation of hydroquinone to 1,2,4-trihydroxybenzene followed by a ring-fission reaction. The second pathway of hydroquinone degradation is less common in nature. In this pathway, hydroquinone ring is directly cleaved and the generated semialdehyde is oxidized to maleylacetate.[63,64] The first aerobic branch has been characterized in prokaryotes and fungi; meanwhile, the second is exclusive to prokaryotic organisms.[60] The anaerobic biodegradation of hydroquinone is a less frequent process in nature, mainly restricted to a few bacteria. It involves the conversion of hydroquinone to benzoate with an intermediate carboxylation, and activation of the products by their linkage to acetyl-CoA (Figure 3).[60]

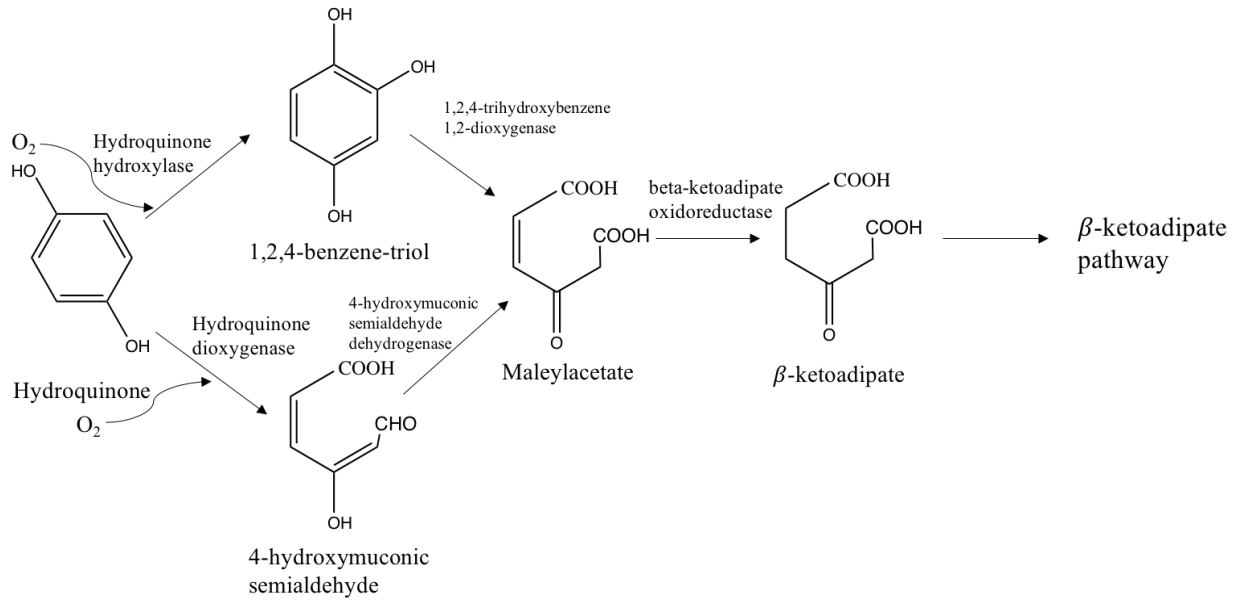



**Figure 2**. Two different branched pathways for the biodegradation of hydroquinone under aerobic conditions. Complete mineralization involves the $\beta$-ketoadipate pathway.[60]

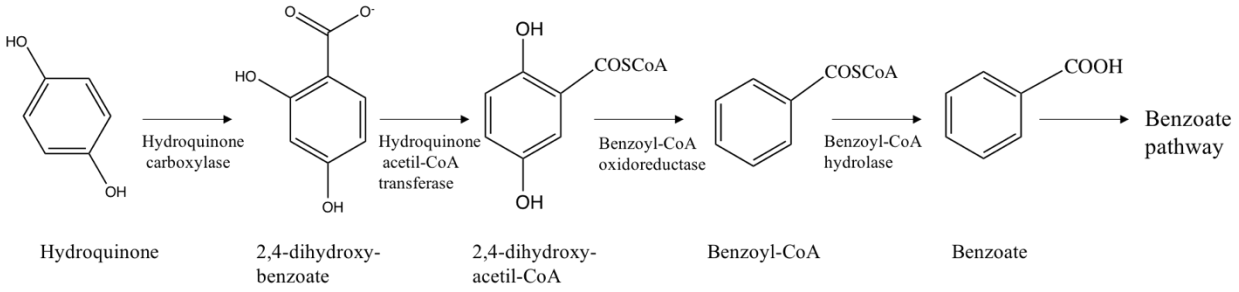



**Figure 3.** Initial step in the anaerobic pathway for the metabolism of hydroquinone. Complete mineralization involves the benzoate pathway.[60]

## 2.2.3 Catechol toxicity and biodegradation

Catechol, also known as pyrocatechol or 1,2-dihydroxybenzene, is a derivative of benzene and a phenolic compound. It is widely used in photographic developer, lubricating oil, polymerization inhibitor and pharmaceuticals.[65,66] Catechol is also a toxic and persistent water pollutant in the environment. The International Agency for Research on Cancer (IARC) has classified catechol

in terms of a carcinogenic risk to humans as Group 2B.[65] Additionally, catechol is either toxic or lethal to fish at concentrations of 5 - 25 mg/L.[67]

Several groups of aerobic bacteria are capable of using catechol. Under aerobic conditions, the ortho and meta cleavage pathways (Figure 4[68]) are two typical pathways for metabolizing the catechol compound[69]. In an aerobic degradation process, most of the microorganisms typically utilize the ortho pathway[57]. There have been several reports on the biodegradation of catechol by some microbial strains under aerobic conditions such as *Pseudomonas putida*[67], and the fungi *Aspergillus awamori*[70] and *Candida parapsilopsis*[71].

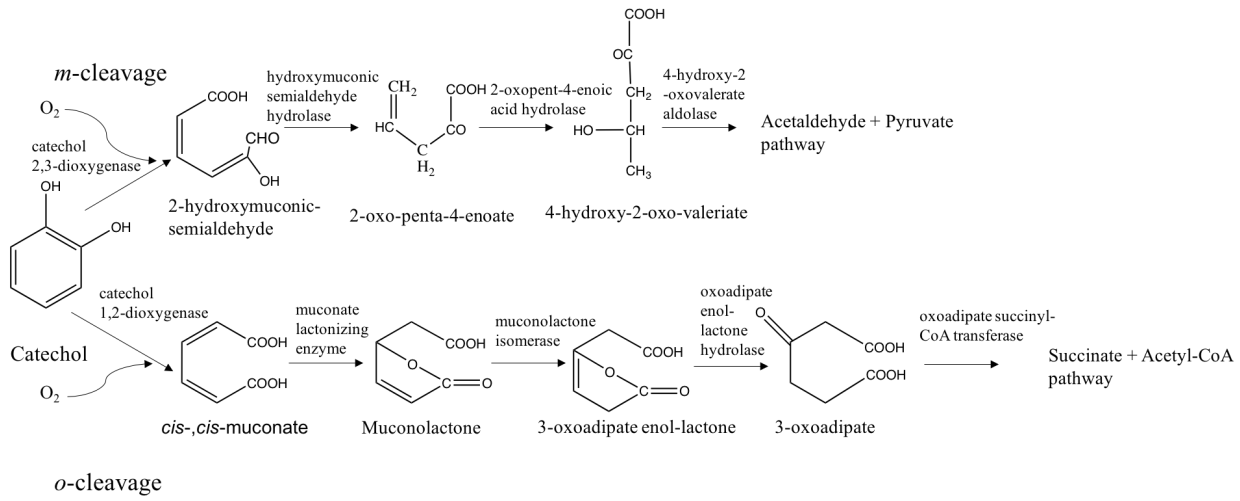



**Figure 4.** The two alternative pathways known for aerobic degradation of catechol: *o*- and *m*-cleavage.[68] Complete mineralization involves the acetaldehyde + pyruvate pathway and succinate + acetyl-CoA pathway.

Some researchers have also studied the degradation of catechol in anaerobic bioreactors including upflow fixed film-fixed bed bioreactors[72] and upflow anaerobic sludge blanket (UASB) bioreactors[66]. In the upflow fixed film-fixed bed reactor, maximum substrate removal of 93.11% was obtained at the loading of 4.79 kg catechol/$m^3$ void vol/day and when the loading

increased to 7.39 kg catechol/$m^3$ vol/day the removal percentage was 57%.[72] Due to the greater inhibitory effect of catechol on anaerobic microbial metabolism, aerobic biological methods are conventionally preferred to anaerobic biological methods for the treatment of catechol-containing wastewater.[69]

### 2.2.4 Resorcinol toxicity and biodegradation

Resorcinol, benzene-1,3-diol, is one of the intermediates in the metabolic pathway during the biodegradation of numerous aromatics.[73] Resorcinol is an irritant for skin, eyes and mucous membranes. Resorcinol is usually utilized to produce dyes, plastics and synthetic fibers. As a result, resorcinol is extensively present in the effluents generated during its production as well as during its usage.[74] Resorcinol is hard to remove in wastewaters due to its high toxicity and low biodegradability.[75,76]

The metabolism of resorcinol by bacteria under aerobic conditions was studied by Peter and Douglas. The major pathway is shown in Figure 5.[77]

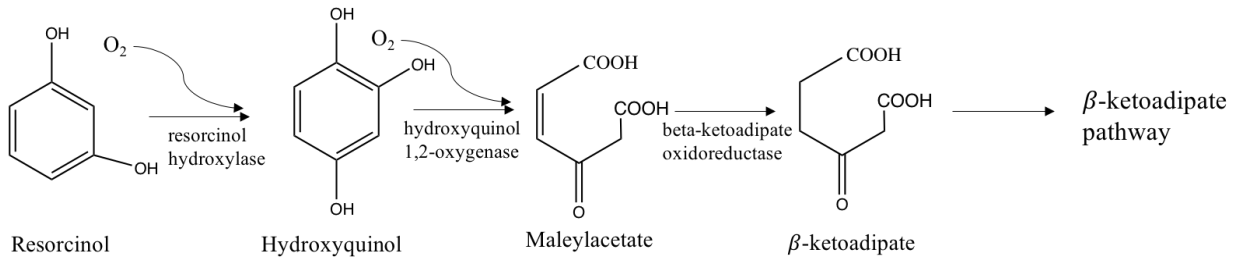
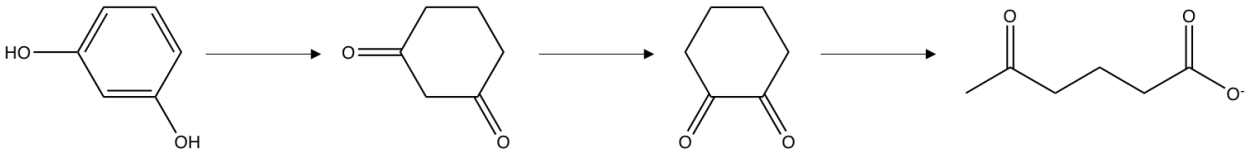



**Figure 5.** Metabolic sequences for resorcinol catabolism used by *Pseudomonas putid*a ORC and *Pseudomonas putida* O1OC.[77] Complete mineralization involves the ß-ketoadipate pathway.

Some researchers have also studied the degradation of resorcinol under anaerobic conditions. Studies reported that resorcinol did not get degraded under methanogenic conditions when the reactors were inoculated with the cow manure as the starting material and continuously fed with the organic household wastes for 140 days.[78] However, Schink and coworkers reported that a fermenting anaerobic bacterium, *Clostridium* strain KN245 is able to degrade resorcinol through the degradation pathway presented in Figure 6[79].




Resorcinol Dihydroresorcinol Cyclohexanedione 5-oxohexanoate

**Figure 6.** Resorcinol degradation pathway of *Clostridium* strain KN245.[79]

### 2.2.5 Overlaps in phenol, hydroquinone, catechol and resorcinol degradation pathways

From the degradation pathways (Figure 1), we can see catechol is on one of the phenol degradation paths and one of the phenol degradation pathways and resorcinol degradation pathways share a common enzyme phenol 2-monooxygenase.

# CHAPTER 3: MATERIALS AND METHODS

## *3.1 Reactor set-up and operation*

### 3.1.1 Reactor system

To first enrich for acetate-utilizing granules, two 3.5L granular sequencing batch reactors (GSBRs) were constructed of clear PVC pipes, with an internal diameter of 2.756 inches (Figure 7). Air was introduced via a fine bubble aerator at the bottom of the reactors (4 L/min) to mix and aerate the reactors during the aeration phase. Media was added by peristaltic pumps (see media section below).[8] One of the GSBRs was inoculated with return activated sludge (1.5 L) from Ithaca Area Wastewater Treatment Facility (Ithaca, NY) and the other one was inoculated with return activated sludge (700 mL) from Ithaca Area Wastewater Treatment Facility (NY), anaerobic digester biomass (500 mL) from Ithaca Area Wastewater Treatment Facility (NY), three pure cultures of methanotrophic bacteria (197.5 mL), and Enhanced Biological Phosphorus Removal (EBPR) sludge from a full scale treatment plant in Pennsylvania (300 mL settled sludge).

The reactors were operated in successive cycles of around 3 hours total, divided into a set sequence: 10 minutes feeding from the bottom of the reactor, 45 minutes anaerobic phase, 110 minutes aerobic phase, 2 minutes settling phase and 10 minutes effluent discharging phase (discharging through ports ½ way up the wall of reactors). Dissolved oxygen was measured using a dissolved oxygen probe (YSI instruments).Hydraulic retention time (HRT) was around 6 h and substrate load, measured in chemical oxygen demand (COD), was 2.4 kg $m^{-3}day^{-1}$.[8] After stable granules were formed (based on visual inspection of the shape and size of bioflocs using a

back-lit colony counter), the biomasses in the two reactors were combined and redistributed between the two reactors. There was no intentional solids wasting so the only biomass losses were in the effluent. Suspended solids levels were not monitored in the effluent. Therefore, solids retention times could not be calculated in this work.

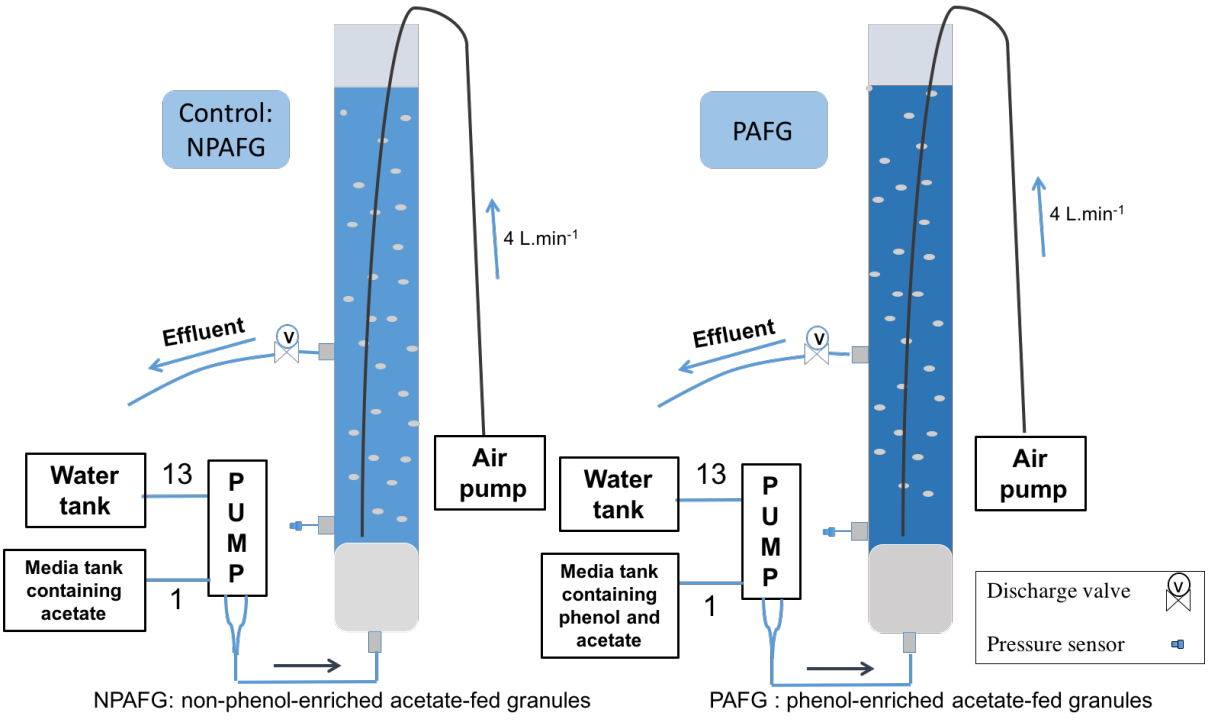



**Figure 7.** Reactor configurations

### 3.1.2 Experiment timeline

There were six stages in the experiment (Figure 8 and Table 1).

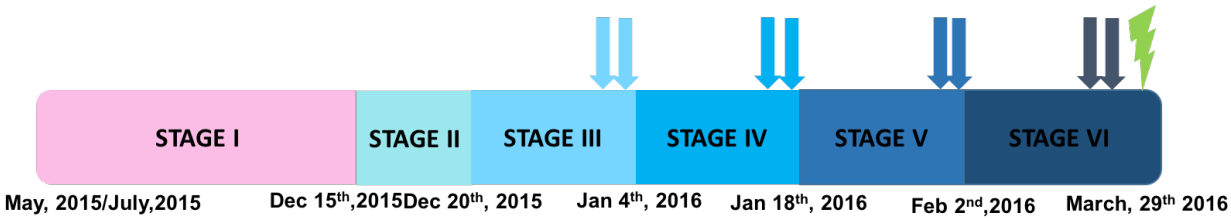



**Figure 8.** Experiment timeline flow chart. The detailed experiment timeline is shown in Table 1. The bold arrows represent removal tests of hydroquinone, catechol and resorcinol individually at the corresponding phenol enrichment concentrations. The lighting bolt represents the removal test of phenol and three

dihydroxybenzenes in a mixture at 50 mg/L each.

**Table 1.** Detailed experiment timeline

| Stage Number | Date | Stage details |
|---|---|---|
| **Stage 1** | May/July,2015-Dec 15th, 2015 | Cultivation of stable aerobic granules using acetate-containing synthetic wastewater (Total COD: 600 mg/L) (Throughout condition for control reactor: non-phenol- enriched acetate-fed granules reactor) |
| **Stage 2** | Dec 15th, 2015-Dec 20th,2015 | Enrichment with: 10 mg/L phenol + acetate remainder (Total COD: 600 mg/L) |
| **Stage 3** | Dec 20th,2015-Jan 4th,2015 | Enrichment with: 25 mg/L phenol + acetate remainder (Total COD: 600 mg/L)<br>After the phenol-enriched reactor had a good final removal percentage of phenol at 25 mg/L concentration, removal tests of hydroquinone, catechol and resorcinol individually at 25 mg/L were performed twice (two days before Stage 4). |
| **Stage 4** | Jan 4th,2016-Jan 18th,2016 | Enrichment with: 50 mg/L phenol + acetate remainder (Total COD: 600 mg/L)<br>After the phenol-enriched reactor had a good final removal percentage of phenol at 50 mg/L concentration, removal tests of hydroquinone, catechol and resorcinol individually at 50 mg/L were performed twice (two days before Stage 5). |
| **Stage 5** | Jan 18th,2016-Feb 2nd,2016 | Enrichment with: 75 mg/L phenol + acetate remainder (Total COD: 600 mg/L)<br>After the phenol-enriched reactor had a good final removal percentage of phenol at 75 mg/L concentration, removal tests of hydroquinone, catechol and resorcinol individually at 75 mg/L were performed twice (two days before Stage 6). |
| **Stage 6** | Feb 2nd,2016-March 29th,2016 | Enrichment with: 100 mg/L phenol + acetate remainder (Total COD: 600 mg/L)<br>After the phenol-enriched reactor had a good final removal percentage of phenol at 100 mg/L concentration, removal tests of hydroquinone, catechol and resorcinol individually at 100 mg/L were performed twice.<br>At the end, a removal test of three dihydroxybenzenes and phenol mixture at 50mg/L each was performed. |

### 3.1.3 Sampling points during one cycle

There were 7 sampling time points in one cycle (Table 2) to capture the dynamics of removal during anaerobic and aerobic portions of the cycles.

**Table 2.** Sampling time points during one cycle

| | |
|---|---|
| **1** | Influent (taken from influent tubing line) |
| **2** | End of feeding phase (taken from the reactor) |
| **3** | Middle of anaerobic phase (taken from the reactor) |
| **4** | End of anaerobic phase (taken from the reactor) |
| **5** | Aeration phase 2200s (taken from the reactor) (after 1/3 of aeration time) |
| **6** | Aeration phase 4400s (taken from the reactor) (after 2/3 of aeration time) |
| **7** | Effluent (taken from effluent tubing line) (after full aeration time) |

### 3.1.4 Media

During stage one and throughout for the control reactor (NPAFG): the composition of the influent media was based on literature recipes for lab-scale GSBRs.[8] Concentrated acetate media contained: NaAc 10.77 g/L, $MgSO_4 \cdot 7H_2O$ 1.25 g/L, KCl 0.492 g/L, $NH_4Cl$ 2.66 g/L, $K_2HPO_4$ 1.35 g/L, $KH_2PO_4$ 0.4 g/L, and trace element solution 10 mL/L (according to Vishniac and Santer).[80] For both reactors 125 mL concentrated media per cycle was dosed together with 1625 mL tap water to achieve a COD in the influent of 600 mg COD/L. The organic loading rate, measured in chemical oxygen demand (COD), was 2.4 kg $m^{-3}$ $day^{-1}$. From stage two to stage six, the concentration of NaAc decreased gradually in the experimental reactor (PAFG) and phenol (Alfa Aesar, 99%) was increased correspondingly to maintain total COD at 600 mg COD/L.

## *3.2 Analytical procedures*

### 3.2.1 Sampling procedure

At sampling times, 15 mL samples were centrifuged in 15-mL plastic centrifuge tubes (Corning®) at 4000 *x g* for 10 minutes at room temperature and then filtered (Acrodisc® 25-mm syringe filter with 1-μm pore size membrane). Then 2-mL sub-samples were distributed into 2

mL vials (Fisherbrand™ 8-425 amber screw vial) used for HPLC analysis and into COD vials for soluble COD analysis. A flowchart is shown in Figure 9. Additionally, occasionally granule samples were visualized for size/shape monitoring by taking a 15-mL sample into a petri-dish and illuminating from behind using a colony-counter light source.

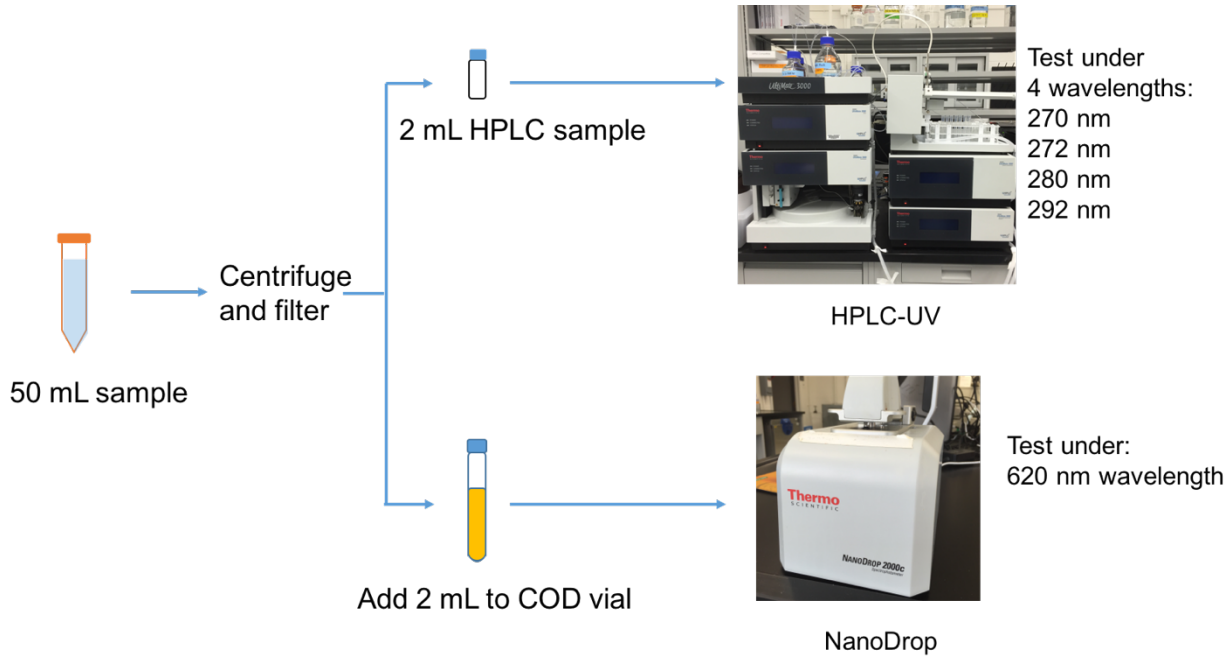



**Figure 9.** Sampling procedure flow chart for HPLC and soluble COD samples.

### 3.2.2 Hydroquinone, catechol, resorcinol and phenol measurements

Aromatic-compounds analysis was performed using a high-performance liquid chromatography coupled to a UV detector (HPLC-UV). HPLC-UV used an Ultimate 3000 HPLC System (Thermo Scientific) comprising a Solvent Rack (SR-3000), HPLC Pump (LPG-3400SD), Autosampler (WPS-3000SL), Column Compartment (TCC-3000SD) and Variable Wavelength Detector (VWD-3400RS). Separations were carried out on a Sonoma C18 5-µm (100 Å Pore Size) HPLC Column (250 × 4.6 mm), eluted with mixtures of water (solvent A) and acetonitrile

(solvent B) (VWR®, HPLC Grade) according to the program: 0 - 10 min, 15% B (isocratic); 10 - 23 min, 15 - 100% B (linear gradient); 23 - 30 min, 15% B (isocratic) (see Appendix). The chromatographic conditions were flow rate 0.8 mL $min^{-1}$ and injection volume 20 µL.[81] Each measurement was carried out with the detector monitoring four wavelengths at the same time: 270 nm, 272 nm, 280 nm and 292nm[72], which were the maximum absorption wavelengths for phenol, catechol, resorcinol, hydroquinone, respectively.[81] The retention times and maximum absorption wavelengths were previously determined by injecting 100 mg/L phenol (Alfa Aesar®, 99%), catechol (Alfa Aesar®, 99%), resorcinol (Alfa Aesar®, 99%) and hydroquinone (Alfa Aesar®, 99%) standard solutions individually into HPLV-UV followed by a spectral scan. Linear standard curves were generated from pure chemicals under eight different concentrations: 0 mg/L, 0.5 mg/L, 1 mg/L, 10 mg/L, 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L (see Appendix). Standards were processed the same way as samples to account for any losses during processing (e.g. sorption to filters or tubes).

### 3.2.3 COD measurements

Soluble COD concentrations were determined using COD vials (0 - 1500 mg/L CHEMetric™ COD vials) according to manufacturer's instructions. Briefly, 2 mL of a centrifuged-filtered sample or influent medium was pipetted into the vial, then the cap was secured tightly. The vial was immediately inverted 10 times to mix well. Then the vial was heated at 150 °C for 2 hours and cooled for 15 to 20 min. A 1 mL sample was moved to a cuvette and a NanoDrop (Thermo, 2000c) was used to measure the absorbance of the sample in the cuvette at a wavelength of 620 nm. Linear standard curves were generated from pure acetate (VWR®, Anhydrous ACS Grade)

under 7 different concentrations: 0 mg COD/L, 25 mg COD/L, 50 mg COD/L, 100 mg COD/L, 250 mg COD/L, 500 mg COD/L and 1000 mg COD/L (see Appendix). Standards and samples were handled the same way.

### 3.2.4 TSS and VSS measurements

The total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to Standard Method 2540[82]. The procedure was as follows: a 20 mL sample was taken during the aeration mixing phase and then the water was removed by filtering (Whatman™ 0.7 μm glass filters). The sample was dried for at least 4 hours at 105 °C, until the weight was constant. The weight of the filter and the biomass minus the weight of the filter (its stable weight at 105 °C) is the TSS of the biomass. The sample was then ignited at 550 °C for 15 min, the final weight under 105°C minus the final weight under 550°C divided by 0.02 L is the VSS. Because effluent suspended solids were not measured, solids retention time could not be calculated.

# CHAPTER 4: RESULTS AND DISCUSSION

## *4.1 Basic performance of GSBR*

### 4.1.1 Granule characteristics

During spring and summer of 2015, two GSBRs were inoculated and fed synthetic wastewater with acetate as the sole electron donor. The reactors differed in inocula used but both produced stable granules (based on visual inspection of the shape and size of bioflocs using a back-lit colony counter) within two months of inoculation. The dissolved oxygen levels were monitored and were above 7 mg/L throughout the aerobic phase, but dropped rapidly when airflow stopped. To create replicate GSBRs prior to enriching one with phenol in the media, the granules in the biomass from the two GSBRs were combined and redistributed between the two reactors and feeding resumed as usual. The two GSBRs showed relatively stable soluble COD removals for multiple months (Figure 12) and significant nitrification (70%, data not shown). A picture of the granules at the time of redistribution is shown in Figure 10. As shown, granules were like small balls between 1 and 3 mm. The outer layer is the aerobic zone during aeration and the inner layer may contain anaerobic zones. This feature of granules allows simultaneous nitrification, denitrification and phosphorous removal.[16] The granular structure is compact and deep, which can result in high tolerance to toxic compounds.[83]

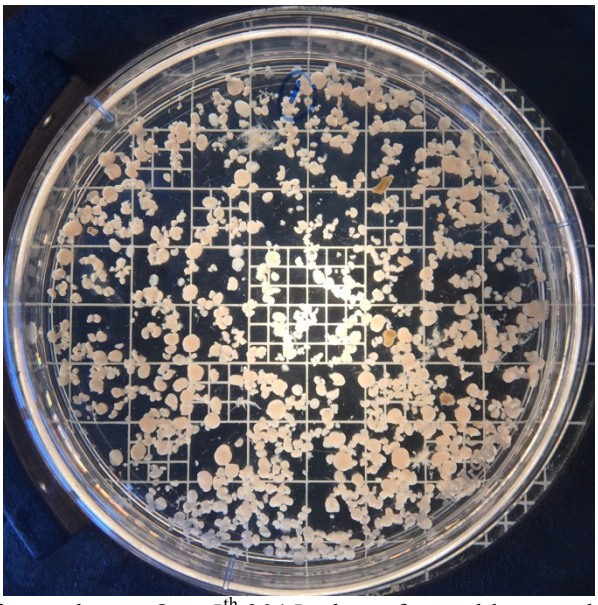


**Figure 10.** A photograph of granules on Oct. 5th 2015 taken after stable granules were formed and prior to phenol enrichment. Most granules ranged from ~ 1 mm to 3 mm. The small grid is 3.3 mm on a side. Large grid squares are 10 mm on a side.

### 4.1.2 Soluble COD removal before phenol enrichment

Before the phenol-enrichment experiment, acetate-containing synthetic wastewater at 600 mg COD/L was used as feed for both of the PAFG reactor and the NPAFG reactor. To compare the PAFG reactor and the NPAFG reactor, the same conditions were applied to both of the reactors for 150 days prior to phenol-enrichment for the PAFG.

Both of the reactors (Figure 11) had a soluble COD removal percentages greater than 90% during one cycle of the GSBR, which indicated that aerobic granules achieved good acetate removal. A two-sample t-test was conducted for the final soluble COD removal percentages between the PAFG reactor and the NPAFG reactor. The p-value for the test is 0.6585, which is higher than the critical value 0.05, so there was no difference between the soluble COD removal

percentages of the two reactors prior to phenol enrichment, and the two reactors could be treated as duplicates prior to the enrichment experiments. Around 20% of the COD was removed from soluble phase during the anaerobic phase, while around 70% of the soluble COD was removed during the aerobic phase (totaling 90% removal over the 3-hour cycle). Over the whole aerobic period (with DO > 7 mg/L), the rate of degradation in both reactors decreased as soluble COD concentrations decreased[55,67,72].

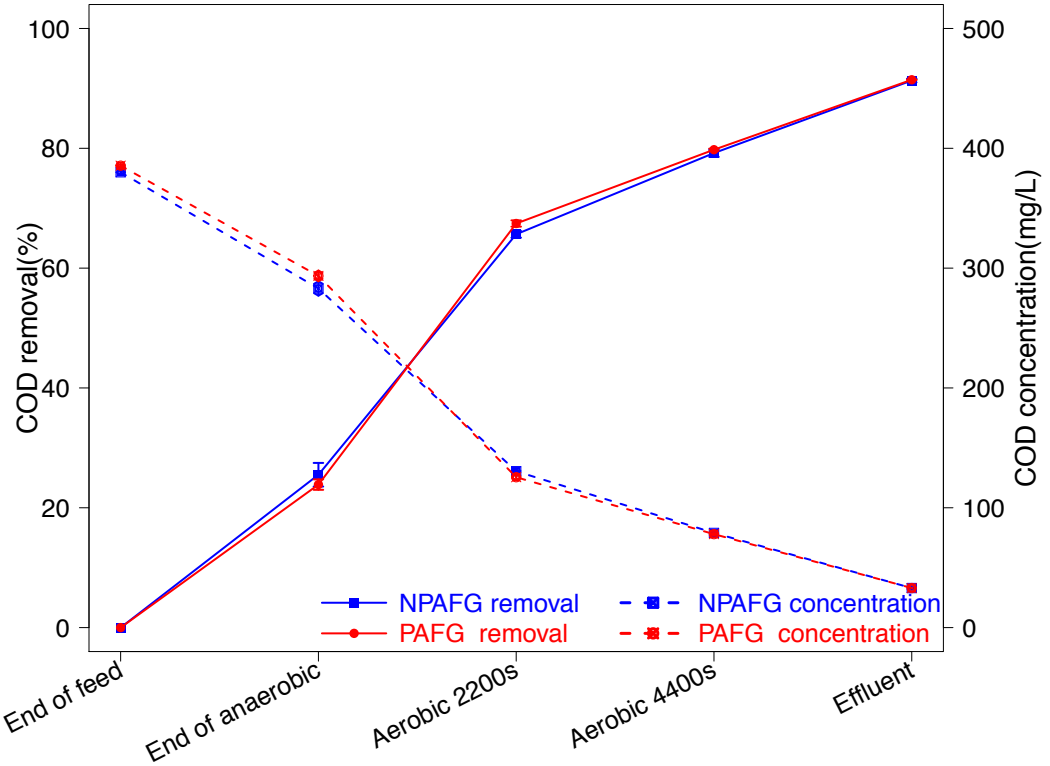



**Figure 11.** Removal of soluble COD at 600 mg COD/L influent concentration after stable granules were formed but before phenol-enrichment. Every point represents the average value of two duplicate experiments. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules. (The time interval between end of feed and end of anaerobic is 2700 seconds. The time interval between end of anaerobic and aerobic 2200s is 2200 seconds. The time interval between aerobic 2200s and aerobic 4400s is 2200 seconds. The time interval between aerobic 4400s and effluent is 2400 seconds). The solid lines are corresponding to the left axis and the dash lines are corresponding to the right axis.

Stable soluble COD removal performance was obtained by both of the reactors over the 6-week period prior to phenol addition (Figure 12). After stable granules were formed, both of the reactors had a soluble COD removal percentages greater than 90% during one cycle of the GSBR.

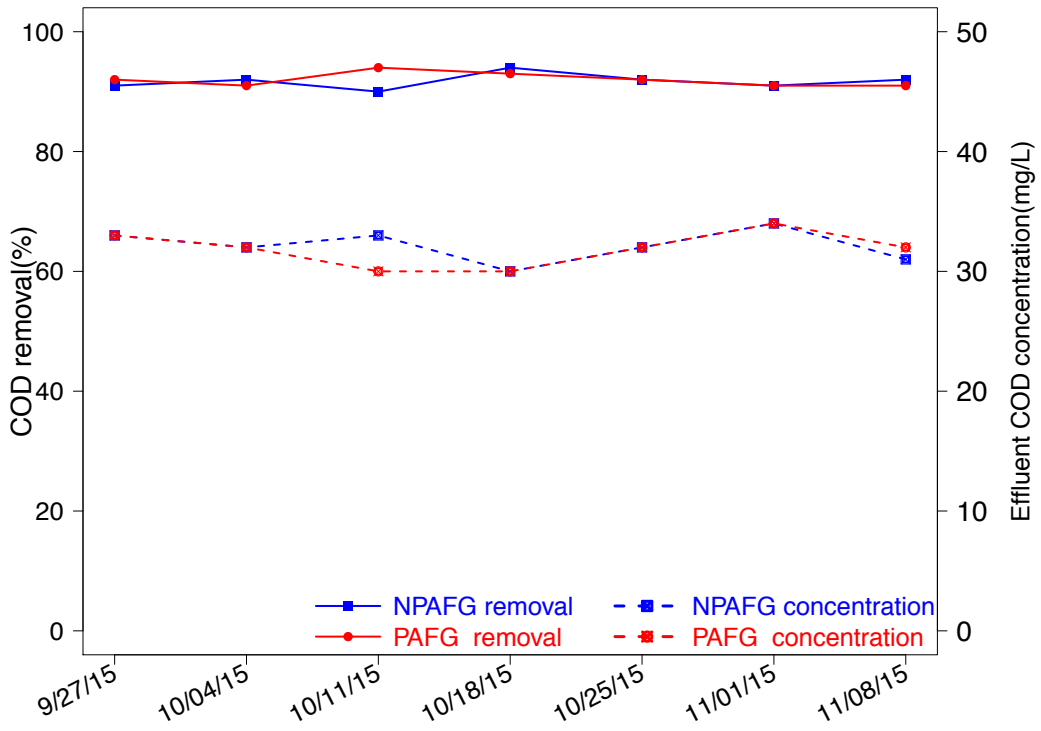



**Figure 12.** Performance of the two reactors on soluble COD removal from 9/27/15 to 11/08/15 after stable granules were formed but before phenol-enrichment. The soluble COD influent concentration was 600 mg COD/L (acetate). NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

### 4.1.3 TSS and VSS results

Before the phenol-enrichment period (Figure 13), the TSS and VSS of PAFG reactor were a little bit higher than those of the NPAFG reactor. Before the phenol-enrichment (on 12/15/15), the

TSS of the NPAFG reactor was around 5.5 g/L and the VSS was around 4 g/L. The TSS of the PAFG reactor was around 5 g/L and the VSS was around 3.8 g/L. However, after the beginning of the phenol-enrichment period (starting 12/15/15), TSS and VSS in the phenol-enriched acetate-fed reactor were lower than those in the non-phenol-enriched acetated-fed reactor for most of the time. This could be attributable to the phenol toxicity, phenol's impact on granule structure (e.g. destabilization) [40].

Dec 15th, Dec 20th, Jan 4th, Jan 18th and Feb 2nd were the days when the phenol concentration was increased. In some cases (Figure 13), the TSS and VSS of the PAFG reactor decreased a little after the phenol increment. The phenol concentration shift-up caused loss of biomass. However, when the granules were adapted to the new phenol concentration, the TSS and VSS of the PAFG reactor increased again to the average value. Overall biomass levels ranged between 4.5 - 5.7 g TSS/L. This is lower than the 8 g TSS/L concentration reported in full scale wastewater treatment plants (using Nereda® technology). However, influent solids in a plant receiving real wastewater would be contributing to TSS level observed at full scale. Also, since SRT cannot be calculated in the current study, a comparison with full scale plants is difficult.

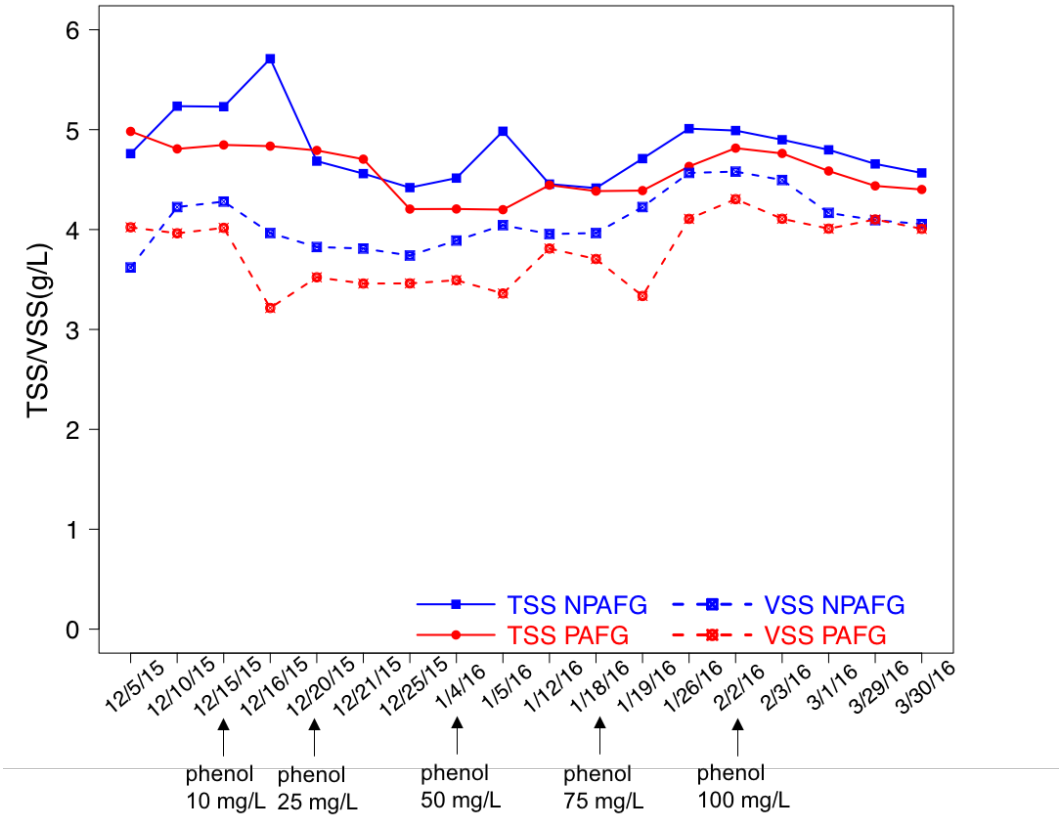



**Figure 13.** TSS (solid lines) and VSS (dashed lines) measurement over time for the reactors in this study. NPAFG: non-phenol-enriched acetate-fed granules, blue. PAFG: phenol-enriched acetate-fed granules, red. Phenol enrichment started on 12/15/15.

## *4.2 Phenol removal results during enrichment of phenol-degraders*

Phenol removal tests were conducted at four phenol-enrichment levels (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L) to determine if the PAFG reactor had adapted to the specific phenol concentration prior to the dihydroxybenzenes tests.

At all four concentrations (Figure 14), the PAFG had good phenol removal performance (90% average) in a cycle. Additionally, the total soluble COD removal percentages were all around

90% (Figure 15). These results indicate the success of the phenol-enrichment. Looking over the time course of one cycle (Figure 14), only around 10% of phenol was taken during the anaerobic phase, while around 80% of the phenol was taken during the aerobic phase. The rate of degradation increased as aerobic phase started, but the rates slowed with time[67,72].

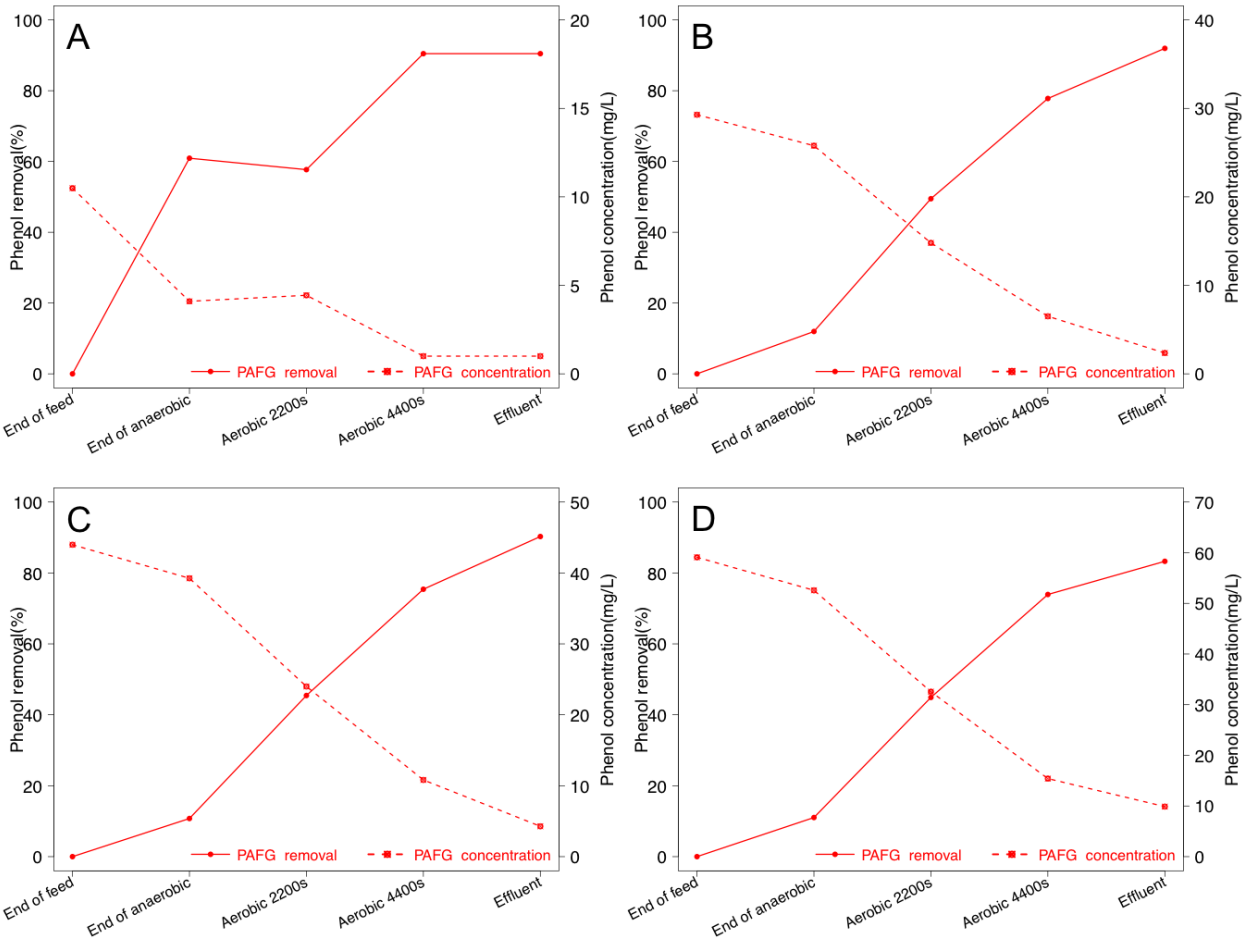



**Figure 14.** Removal of phenol under four different concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L. Note that upon feeding initial concentrations should be 12.5, 25, 37.5 and 50 mg/L respectively due to dilution effects. These data were collected after the reactors had been fed the corresponding concentration for at least 14 days and just before the removal tests for dihydroxybenzenes. All data are for PAFG: phenol-enriched acetate-fed granules. NPAFG was not tested in parallel tests.

Soluble COD removal percentages (Figure 15) were more than 90% during one cycle of PAFG treating phenol-containing synthetic wastewater. The soluble COD removal trend during one

cycle was similar to that of the PAFG reactor when fed only with acetate (reference Figure 11 above which shows this trend): around 25% of the soluble COD was taken during the anaerobic phase, while around 65% of the soluble COD was taken during the aerobic phase. Over time across the whole aerobic period, the rate of degradation in both reactors decreased[67,72].

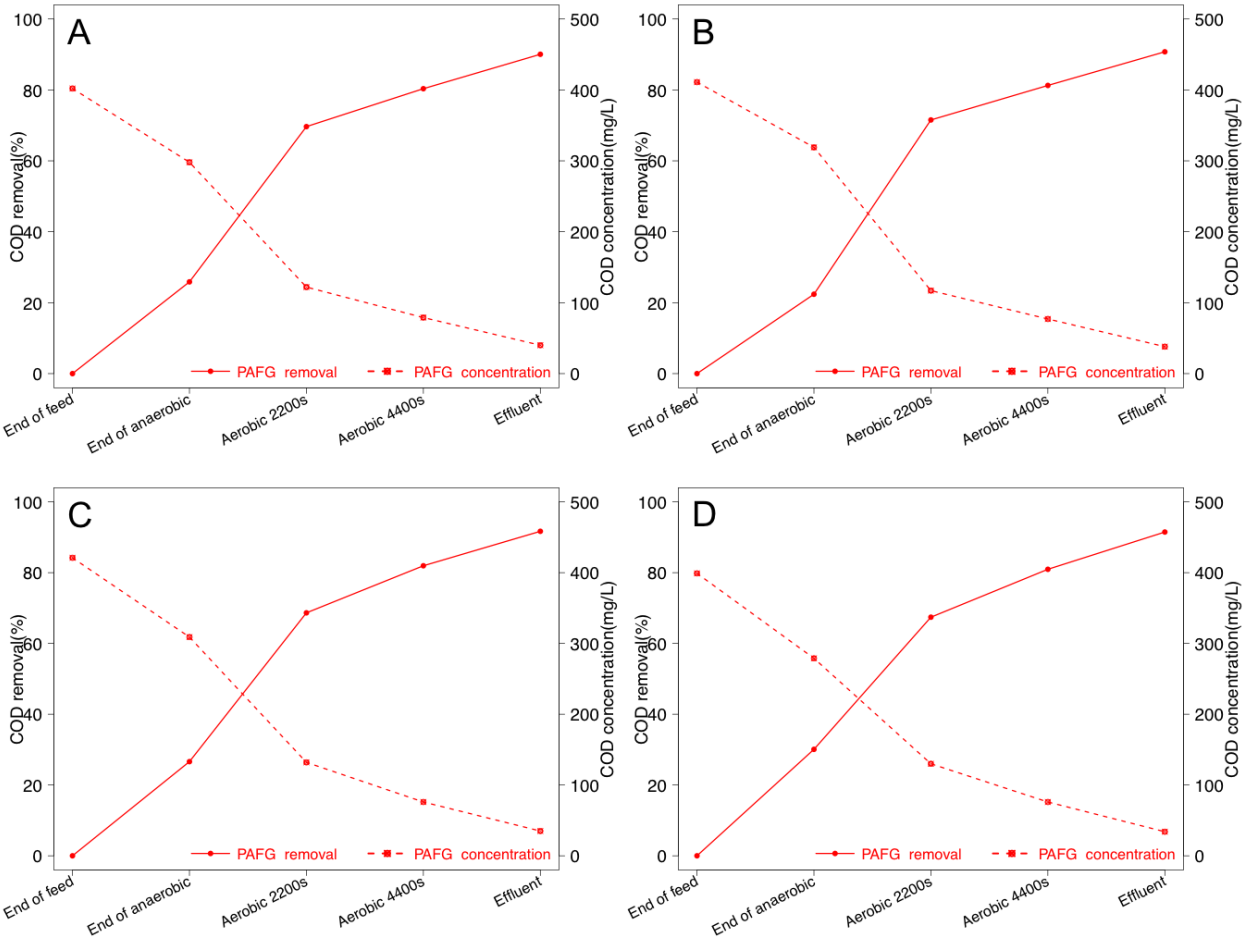



**Figure 15.** The soluble COD removal corresponding to the phenol test in Figure 14 under four different phenol concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L. These data were collected after the reactor had been fed the corresponding concentration for at least 14 days and just before the removal tests for dihydroxybenzenes. The influent soluble COD concentration was 600 mg/L before dilution into the reactor. PAFG: phenol-enriched acetate-fed granules.

## *4.3 Hydroquinone, catechol, resorcinol results*

### 4.3.1 Hydroquinone removal test results

Hydroquinone removal tests were conducted under each of four phenol-enrichment levels (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L) after the PAFG reactor had been enriched under 10 mg/L phenol concentration for 6 days and under 25 mg/L, 50 mg/L 75 mg/L and 100 mg/L for 14 days each.

Under four hydroquinone concentrations (Figure 16) the two reactors all had hydroquinone removal percentages greater than 80% in one cycle. However, the PAFG reactor had slightly higher final hydroquinone removal percentage than the NPAFG reactor. Only under 75 mg/L and 100 mg/L hydroquinone concentrations (Table 3), the p-values of the t-tests, 0.0042 and 0.0338, were smaller than critical p-value 0.05, which means the differences between the final hydroquinone removal percentages of the two reactors were significant. So the phenol-enrichment did improve the hydroquinone removal under 75 mg/L and 100 mg/L concentrations. However, the absolute improvement is small (84% versus 80% under 75 mg/L and 88% versus 81% under 100 mg/L). With respect to actual mass removal rates, the PAFG and NPAFG reactors removed 14 - 42 and 12 - 34 mg/L per cycle, respectively (Figure 17). Normalized to the VSS levels in the respective reactors these corresponded to specific rates of 4 - 13 and 3 -10 mg hydroquinone $\cdot$ g $VSS^{-1}$ $\cdot$ $cycle^{-1}$ across all hydroquinone concentrations.

Within a cycle around 25% of the hydroquinone (Figure 16) was taken during the hour-long anaerobic phase, while around 60% of the hydroquinone was taken during the aerobic phase. The

rate of removal increased as the aerobic phase started, but the rates leveled off with time. From the end of the first one-third of the aerobic phase to the end of the aerobic phase, especially under 75 mg/L and 100 mg/L hydroquinone concentrations, there was a plateau formed. It is important to note that the plateau levels (around 3 - 10 mg/L) were well above the HPLC method's detection limit. This could be explained by a few mechanisms such as transformation-induced toxicity or accumulation of intermediates (feed-back inhibition). It is unlikely that it represents a thermodynamic limitation for biochemical reactions.

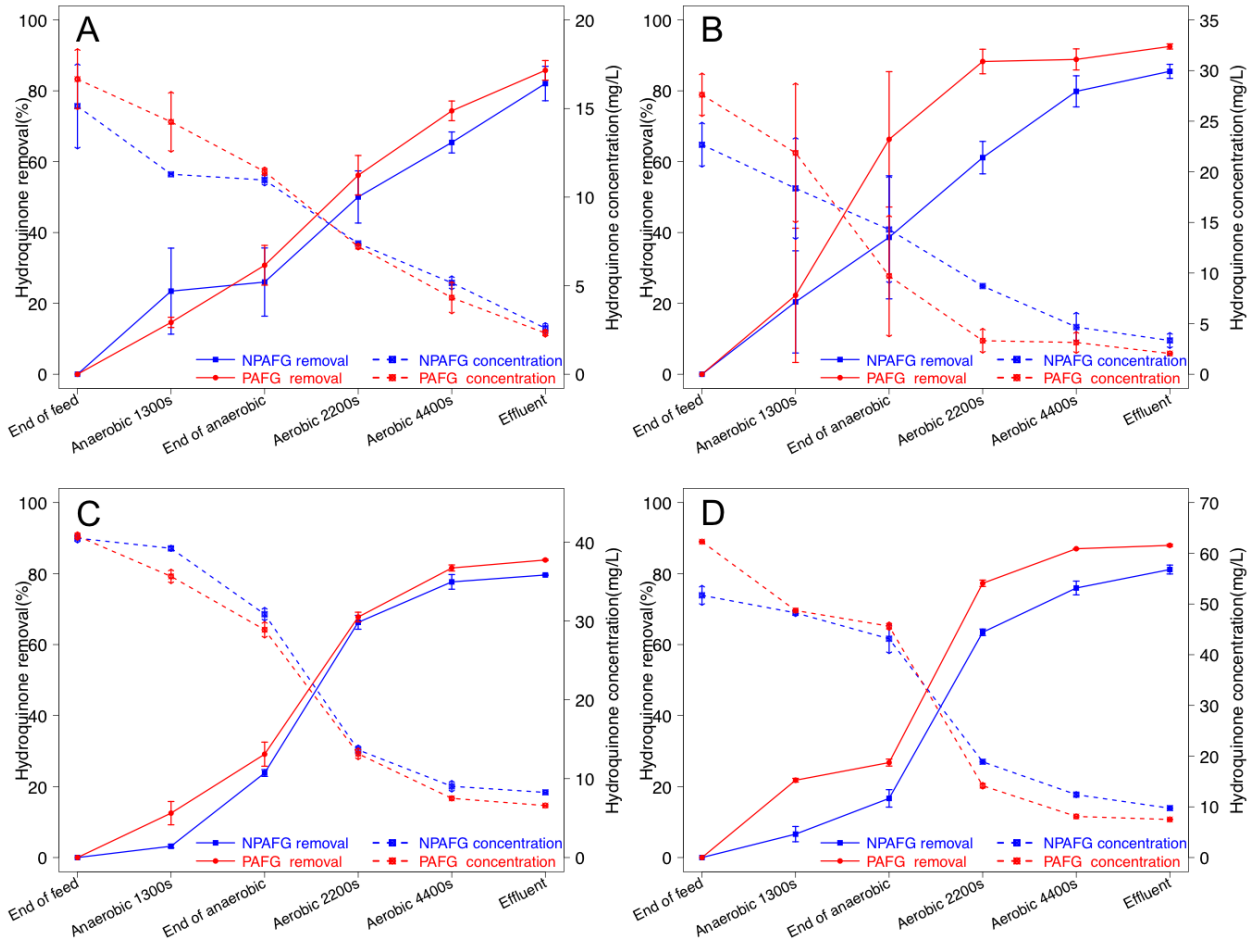



**Figure 16.** Removal of hydroquinone at four different concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L (after the phenol-enrichment under the corresponding concentration). The total soluble COD was 600 mg/L. Every point represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in

the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules. The hydroquinone detection limit was 0.5 mg/L.

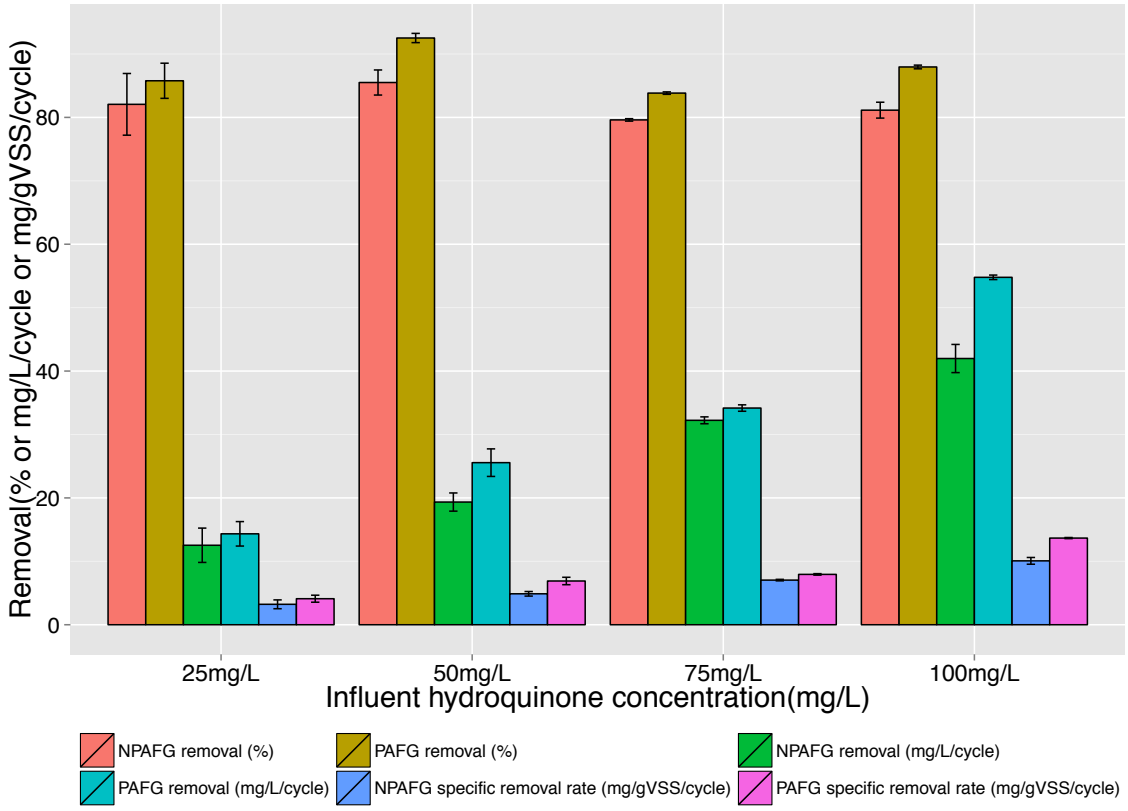



**Figure 17.** Final removal and specific removal rate of hydroquinone at four different influent concentrations. Every bar represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

**Table 3.** Two-sample t-test results of hydroquinone removal between PAFG reactor and NPAFG reactor. Numbers are averages and spread of duplicate tests.

| Hydroquinone concentration in feed (mg/L) | Hydroquinone removal percentages in PAFG | Hydroquinone removal percentages in NPAFG | P-value for hydroquinone removal percentages |
|---|---|---|---|
| 25 | 85.77 ± 3.92 | 82.01 ± 6.88 | 0.5751 |
| 50 | 92.52 ± 1.03 | 85.50 ± 2.80 | 0.0795 |
| 75 | 83.83 ± 0.27 | 79.60 ± 0.28 | **0.0042** |
| 100 | 87.95 ± 0.41 | 81.14 ± 1.77 | **0.0338** |

Both of the reactors (Figure 18) had soluble COD removal percentages greater than 80% during one cycle of treating hydroquinone-containing synthetic wastewater, even in the NPAFG reactor. However, the PAFG reactor still had slightly higher final soluble COD removal percentage than the NPAFG reactor (84% in PAFG compared to 75% in NPAFG). As shown in Table 4, under all four hydroquinone concentrations the p-values of the t-tests were smaller than critical p-value 0.05, which means the differences between the final soluble COD removal percentages of the two reactors were significant. From Figure 18, the overall soluble COD removal trend can be seen as similar to the hydroquinone removal trend.

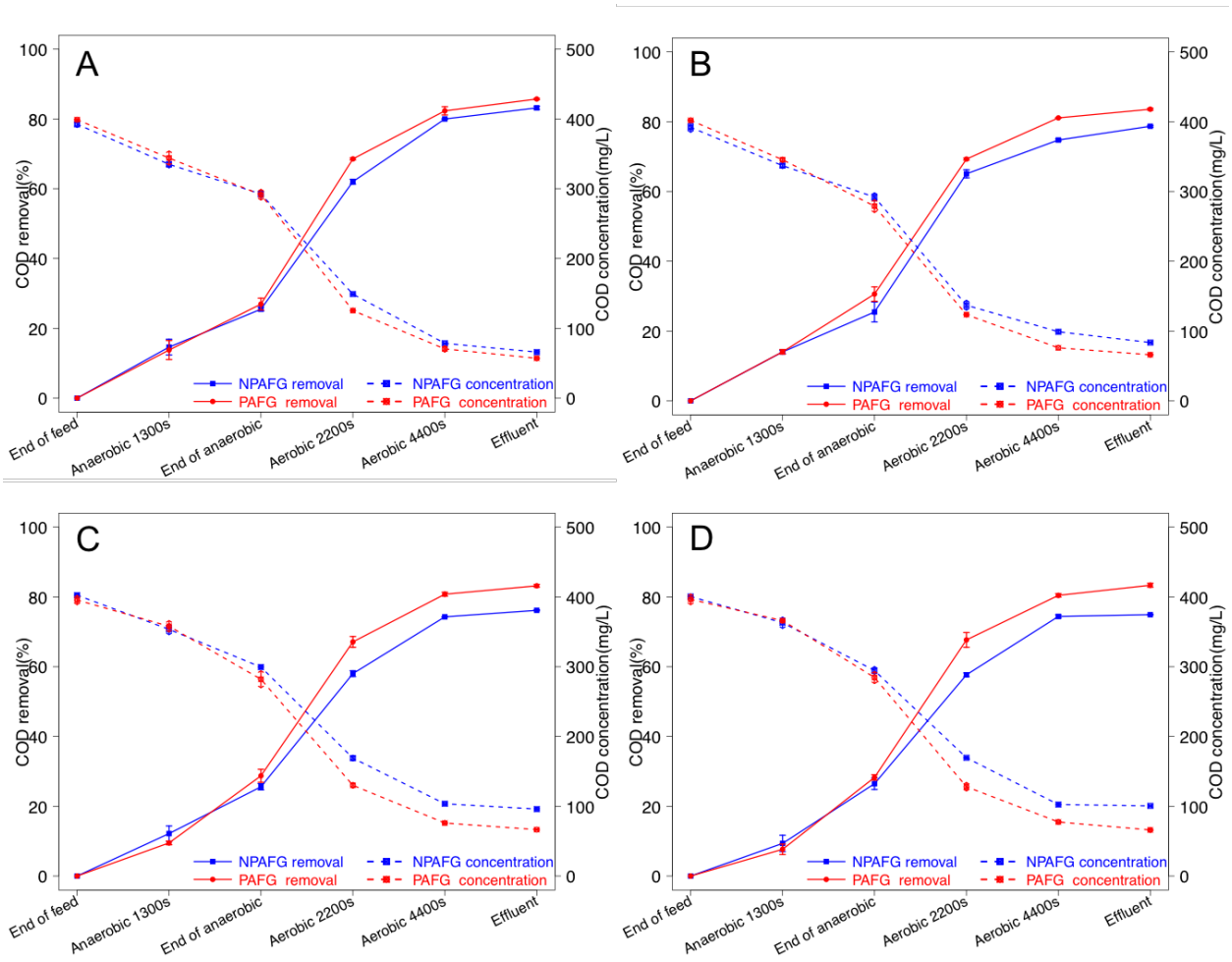



**Figure 18.** The soluble COD removal corresponding to the hydroquinone tests in Figure 16 under four different hydroquinone concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L (after

the phenol-enrichment under the corresponding concentration). The influent soluble COD concentration was 600 mg/L. Every point represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

Because hydroquinone can be readily degraded by activated sludge[84], so the existence of hydroquinone did not weaken the performance of either reactor type very much. Although the differences between the final removal percentages of the two reactors were not large, the differences were statistically significant at higher hydroquinone concentrations (75 mg/L and 100 mg/L). Although the NPAFG reactor was not enriched with phenol or other aromatic compounds, it had a capacity to treat hydroquinone-containing wastewater. The reason is likely that the NPAFG reactor has a diverse microbe community[1]. Additionally, because of granules' compact structure they can tolerate toxic compounds even at high concentration[85]. However, the reason for a plateauing of residual hydroquinone at the higher tested concentrations is unknown and warrants further study.

**Table 4.** Two-sample t-test results of soluble COD removal for hydroquinone tests between PAFG reactor and NPAFG reactor. Numbers are averages and spread of duplicate tests.

| Hydroquinone concentration in feed (mg/L) | Soluble COD removal percentages in PAFG | Soluble COD removal percentages in NPAFG | P-value for soluble COD removal percentages |
|---|---|---|---|
| 25 | $85.71 \pm 0.30$ | $83.18 \pm 0.63$ | **0.0363** |
| 50 | $83.58 \pm 0.41$ | $78.69 \pm 0.36$ | **0.0061** |
| 75 | $83.18 \pm 0.57$ | $76.15 \pm 0.23$ | **0.0038** |
| 100 | $83.34 \pm 0.80$ | $74.91 \pm 0.04$ | **0.0045** |

### 4.3.2 Catechol removal test results

Catechol removal tests were conducted at each of four phenol-enrichment levels (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L) after the PAFG reactor had been enriched with 10 mg/L phenol concentration for 6 days and at 25 mg/L, 50 mg/L 75 mg/L and 100 mg/L for 14 days each.

From Figure 19, for influent with 25 mg/L catechol concentration the reactors both had catechol removal percentages around 70%. However, PAFG reactor had catechol removal percentages around 80% at 50 mg/L, 75 mg/L and 100 mg/L, while NPAFG reactor had removal percentages around 60%. With the increasing of the catechol concentration, the difference in removal between these two reactors increased. The PAFG reactor maintained a good catechol-removal performance, likely due to the continued increase in phenol-degrading populations over the course of the experiment. The performance of the NPAFG reactor became worse with the increasing of the catechol concentration, on the basis of percentage removed. Table 5 shows that differences between the final removal percentages of the two reactors were not really significant at 25 mg/L and 50 mg/L. However, at 75 mg/L and 100 mg/L catechol concentrations, the p-values of the t-tests, 0.0381 and 0.0125, were smaller than the critical p-value 0.05, which means the differences between the final catechol removal percentages of the two reactors were significant. So the phenol-enrichment did improve the catechol removal at 75 mg/L and 100 mg/L concentrations with an absolute improvement of around 20%. With respect to actual mass removal rates, the PAFG and the NPAFG reactors removed 13 - 47 and 12 - 30 mg/L per cycle (Figure 20). Normalized to the VSS levels in the respective reactors these corresponded to specific rates of 4 - 12 and 3 - 7 mg catechol $\cdot$ g $VSS^{-1}$ $\cdot$ $cycle^{-1}$.

Around 20% of the catechol (Figure 19) was removed during anaerobic phase, while around 60% of the catechol was removed during the aerobic phase for the PAFG reactor and around 40% of the catechol was taken for the NPAFG reactor. In both of the reactors, the rate of removal was highest as the aerobic phase started, but the rates leveled off with time. For the NPAFG reactor the aerobic specific degradation rates decreased a lot from the beginning of the aerobic phase to the middle of the aerobic phase, and during the last third of the aerobic phase there were plateaus formed. Especially at 75 mg/L and 100 mg/L catechol concentrations, during the last third of the aerobic phase catechol concentrations remained at the same level in the NPAFG reactor, and this level was well above the detection limit on HPLC (1 mg/L).

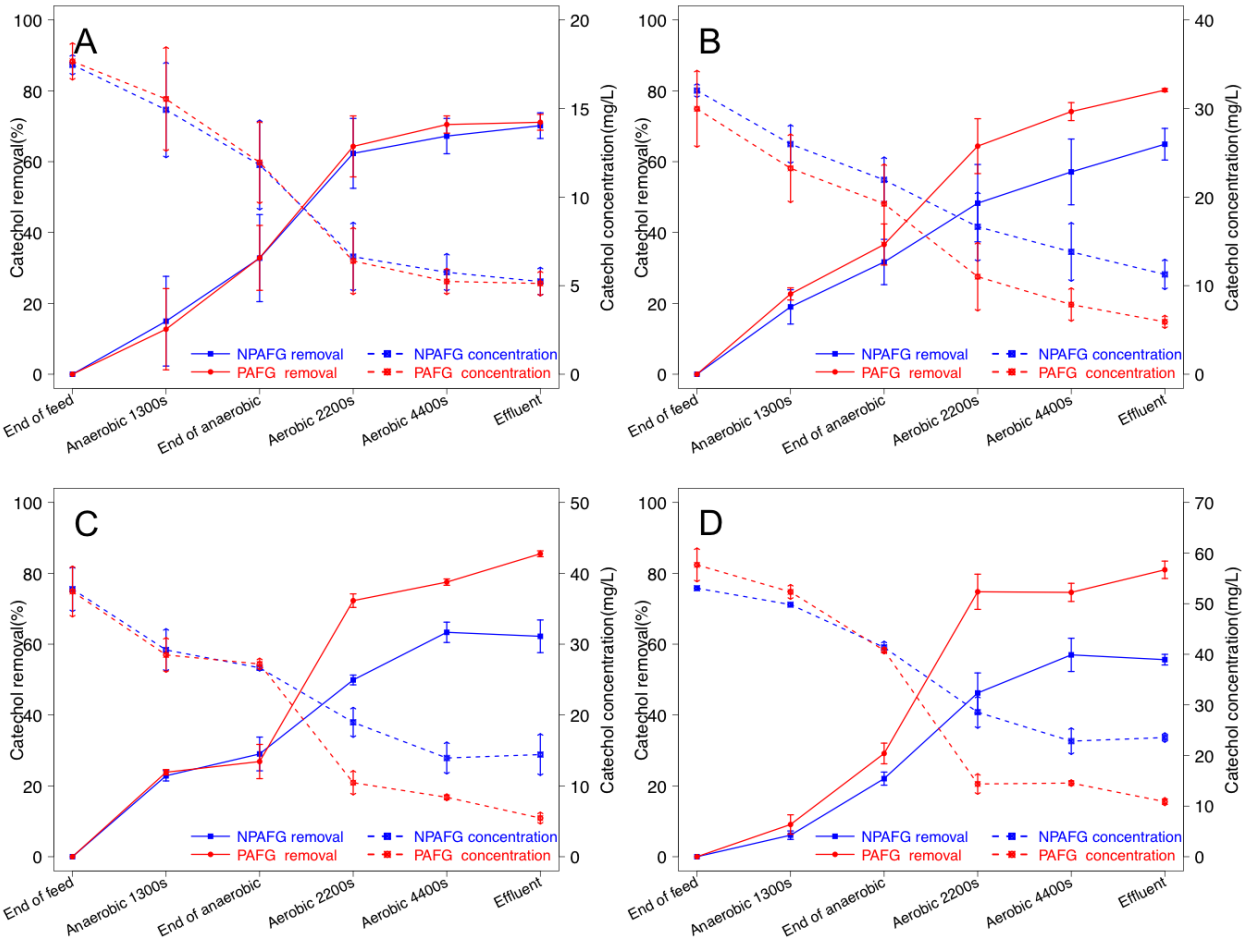


**Figure 19.** Removal of catechol at four different concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L (after the phenol-enrichment under the corresponding concentration). The influent soluble COD was 600 mg/L. Every point represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

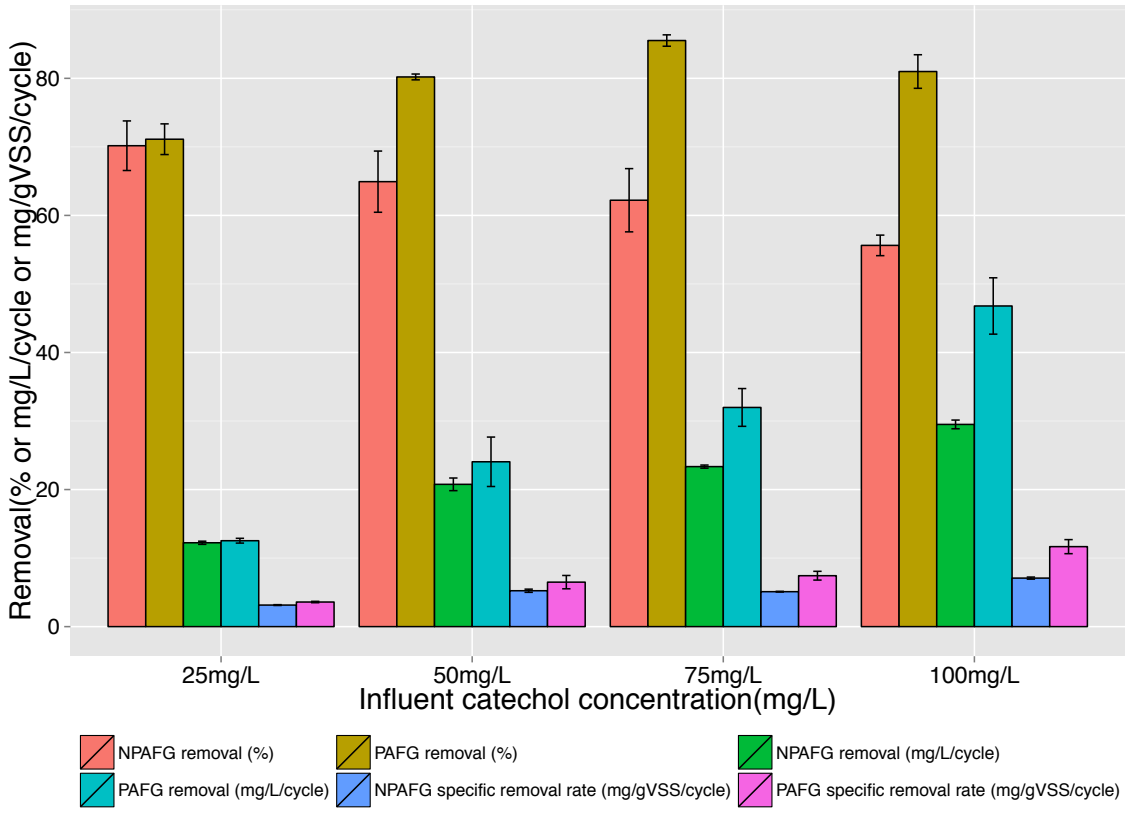



**Figure 20.** Final removal and specific removal rates of catechol at four different influent concentrations. Every bar represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

**Table 5.** Two-sample t-test results of catechol removal between PAFG reactor and NPAFG reactor. Numbers are averages and spread of duplicate tests.

| Catechol concentration in feed (mg/L) | Catechol removal percentages in PAFG | Catechol removal percentages in NPAFG | P-value for catechol removal percentages |
|---|---|---|---|
| 25 | $71.12 \pm 3.17$ | $70.17 \pm 5.12$ | 0.8442 |
| 50 | $80.20 \pm 0.60$ | $64.92 \pm 6.31$ | 0.0764 |

| | | | |
|---|---|---|---|
| 75 | $85.51 \pm 1.17$ | $62.21 \pm 6.52$ | **0.0381** |
| 100 | $80.99 \pm 3.46$ | $55.62 \pm 2.12$ | **0.0125** |

From Figure 21, both of the reactors had soluble COD removal percentages greater than 80% during one cycle of treating catechol-containing synthetic wastewater, except for with 100 mg/L catechol concentration in the NPAFG reactor. The p-values of the t-tests for soluble COD (Table 6) were smaller than critical p-value 0.05 under all four catechol concentrations, which means the differences between the final soluble COD removal percentages of the two reactors were significant. The PAFG reactor had around 8% higher soluble COD removal percentage than the NPAFG reactor. As show in Figure 21, the soluble COD removal trend was similar to catechol removal trend and NPAFG reactor had a plateaus for the soluble COD removal under higher catechol concentrations.

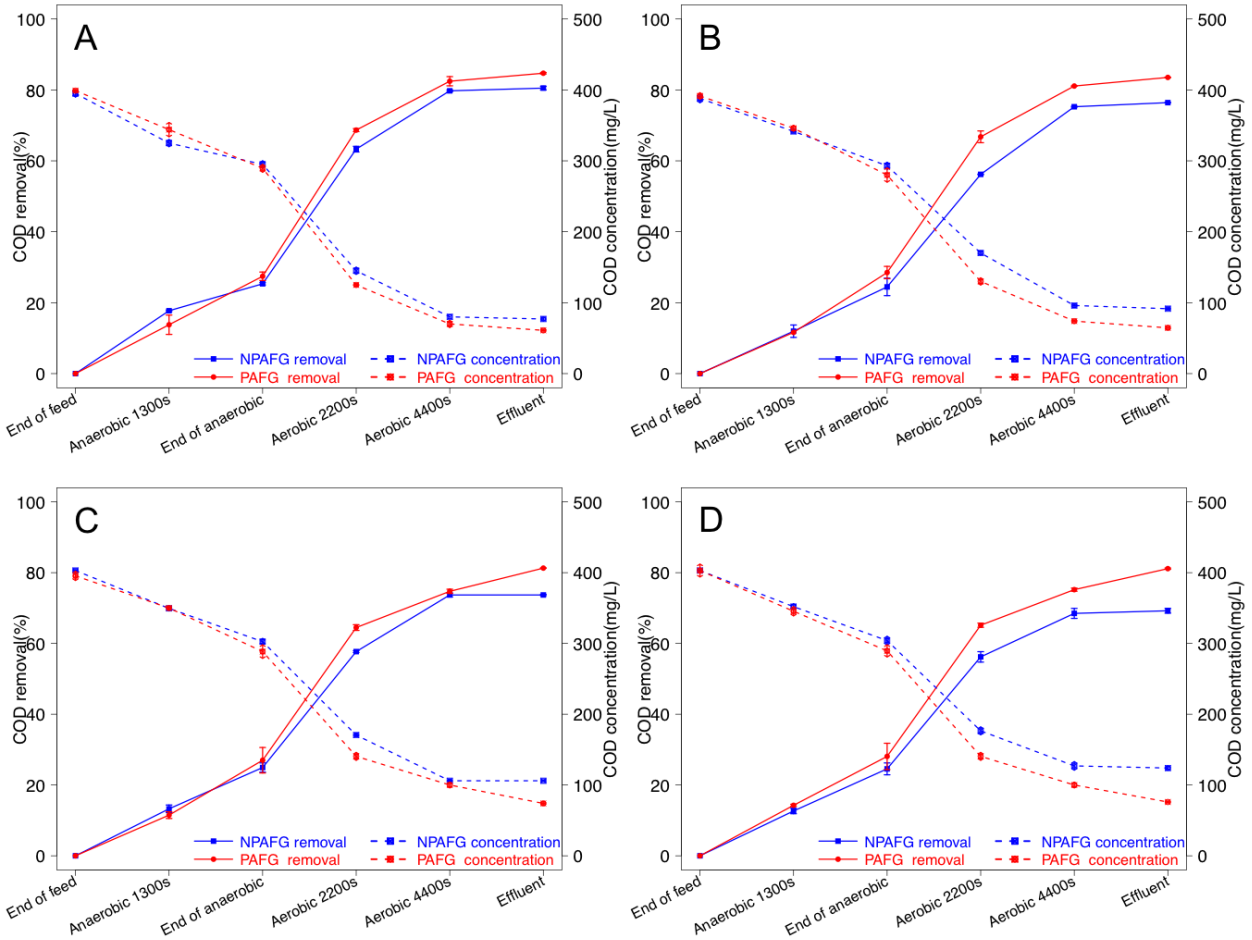



**Figure 21.** The soluble COD removal corresponding to the catechol tests in Figure 19 under four different catechol concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L (after the phenol-enrichment under the corresponding concentration). The influent soluble COD concentration was 600 mg/L. Every point represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

Since catechol is not as readily biodegradable as hydroquinone[72], the NPAFG reactor did not have a good performance for catechol removal, especially at higher catechol concentrations. Therefore, catechol increases did affect the COD removal in NPAFG reactor. Because catechol is one of the intermediate products on one of the phenol degradation pathways, the phenol-enrichment process did enrich the microbes that can degrade phenol through the pathway that contains catechol, which likely resulted in the improvement of the catechol degradation. In the

final stage of the experiment, when phenol comprised around 40% of the influent COD in the PAFG reactor, it is likely that phenol degraders make up a significant percentage of the overall community.

**Table 6.** Two-sample t-test results of soluble COD removal for catechol tests between PAFG reactor and NPAFG reactor. Numbers are averages and spread of duplicate tests.

| Catechol concentration in feed (mg/L) | Soluble COD removal percentages in PAFG | Soluble COD removal percentages in NPAFG | P-value for soluble COD removal percentages |
|---|---|---|---|
| 25 | $84.71 \pm 0.30$ | $80.51 \pm 0.73$ | **0.017** |
| 50 | $83.52 \pm 0.09$ | $76.42 \pm 0.03$ | **8.480E-05** |
| 75 | $81.29 \pm 0.08$ | $73.70 \pm 0.26$ | **0.0006** |
| 100 | $81.14 \pm 0.25$ | $69.23 \pm 0.94$ | **0.0033** |

### 4.3.3 Resorcinol removal test results

Resorcinol removal tests were conducted at each of four phenol-enrichment levels (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L) after the PAFG reactor has been enriched with 10 mg/L phenol concentration for 6 days and under 25 mg/L, 50 mg/L 75 mg/L and 100 mg/L for 14 days each.

From Figure 22, at four resorcinol concentrations, the differences between the resorcinol removal percentages of the two reactors were around 35%. Although the resorcinol concentration was increased to 100 mg/L, the PAFG reactor still maintained a similar removal percentage (all were around 60%, see Figure 24) compared to lower concentrations. In contrast, the performance of the NPAFG reactor became worse with increasing resorcinol concentration. The NPAFG reactor had removal percentages around 25%, and when the resorcinol concentration was increased to 100 mg/L, the removal percentage decreased to 20%. However, when comparing the mass removal rates with increasing resorcinol concentration, even the NPAFG reactor shows

increasing removal rates at higher starting concentrations. With respect to actual mass removal rates, the PAFG reactor removed 10 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (25 mg/L), 19 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (50 mg/L), 28 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (75 mg/L) and 34 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (100 mg/L), while the NPAFG reactor removed 5 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (25 mg/L), 9 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (50 mg/L), 13 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (75 mg/L) and 12 mg resorcinol · $L^{-1}$ · $cycle^{-1}$ (100 mg/L) (Figure 23). Normalized to the VSS levels in the respective reactors these corresponded to specific rates of 2.8 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (25 mg/L), 5.2 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (50 mg/L), 6.6 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (75 mg/L) and 8.5 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (100 mg/L) in the PAFG reactor, and 1.2 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (25 mg/L), 2.2 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (50 mg/L), 2.9 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (75 mg/L) and 2.9 mg resorcinol · g $VSS^{-1}$ · $cycle^{-1}$ (100 mg/L) in the NPAFG reactor.

As shown in Table 7, under the four concentrations the p-values of the t-tests were all smaller than the critical p-value 0.05, which means the differences between the final resorcinol removal percentages of the two reactors were all significant. So the phenol-enrichment did improve the resorcinol removal and the absolute improvement was around 35 percentage points (60% in PAFG versus 25% in NPAFG). And as shown in the Figure 22, around 15% of the resorcinol was taken during anaerobic phase, while around 45% of the resorcinol was taken during the aerobic phase for PAFG and around 10% of the resorcinol was taken for NPAFG. In both of reactors, the rate of degradation was increased as they went from anaerobic to aerobic conditions, and during the full aerobic phase the rate of degradation barely changed[15,55]. The rates of degradation in the PAFG reactor were larger than those of the NPAFG reactor.

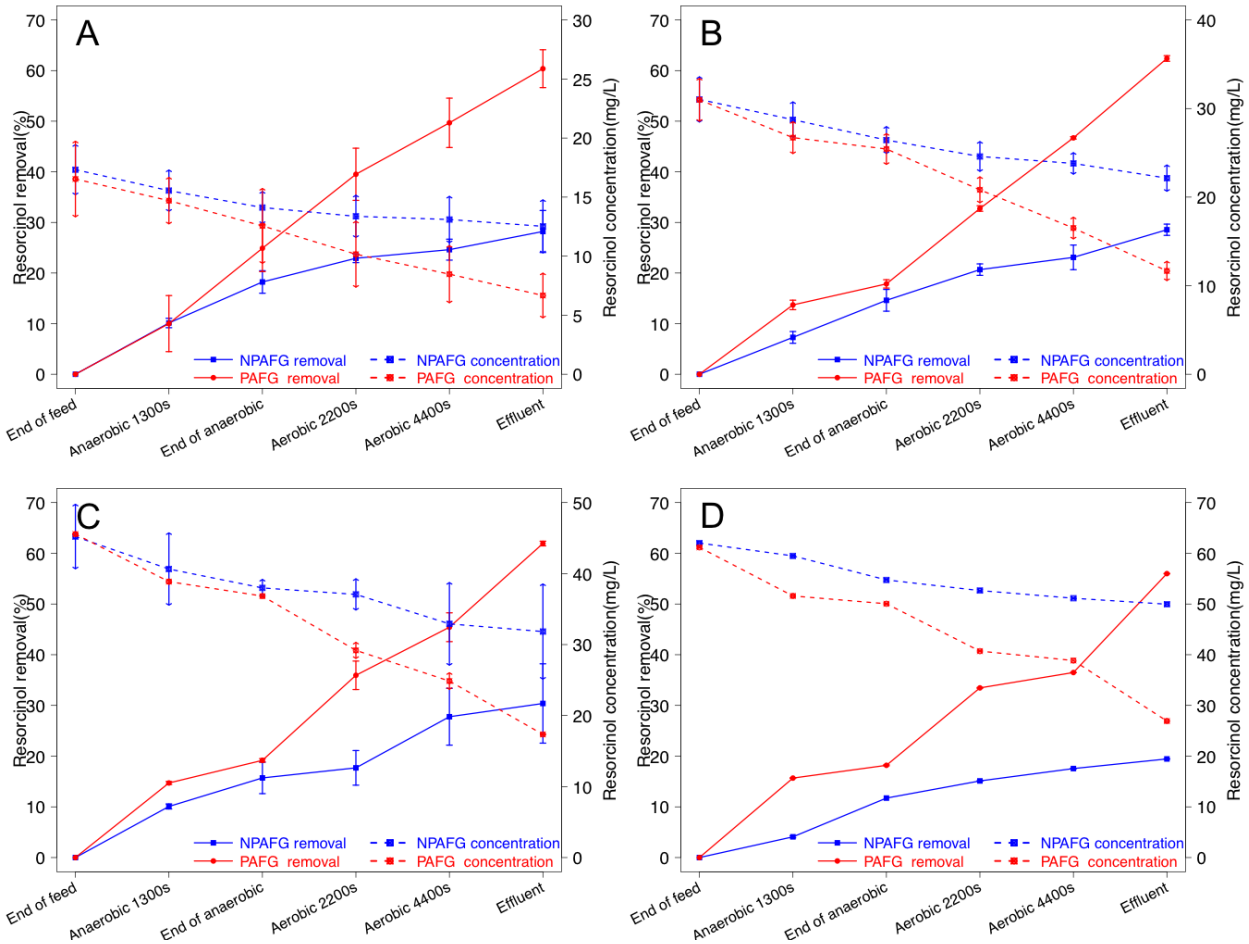



**Figure 22.** Removal of resorcinol at four different concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L (after the phenol-enrichment under the corresponding concentration). The influent soluble COD was 600 mg/L. Every point represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

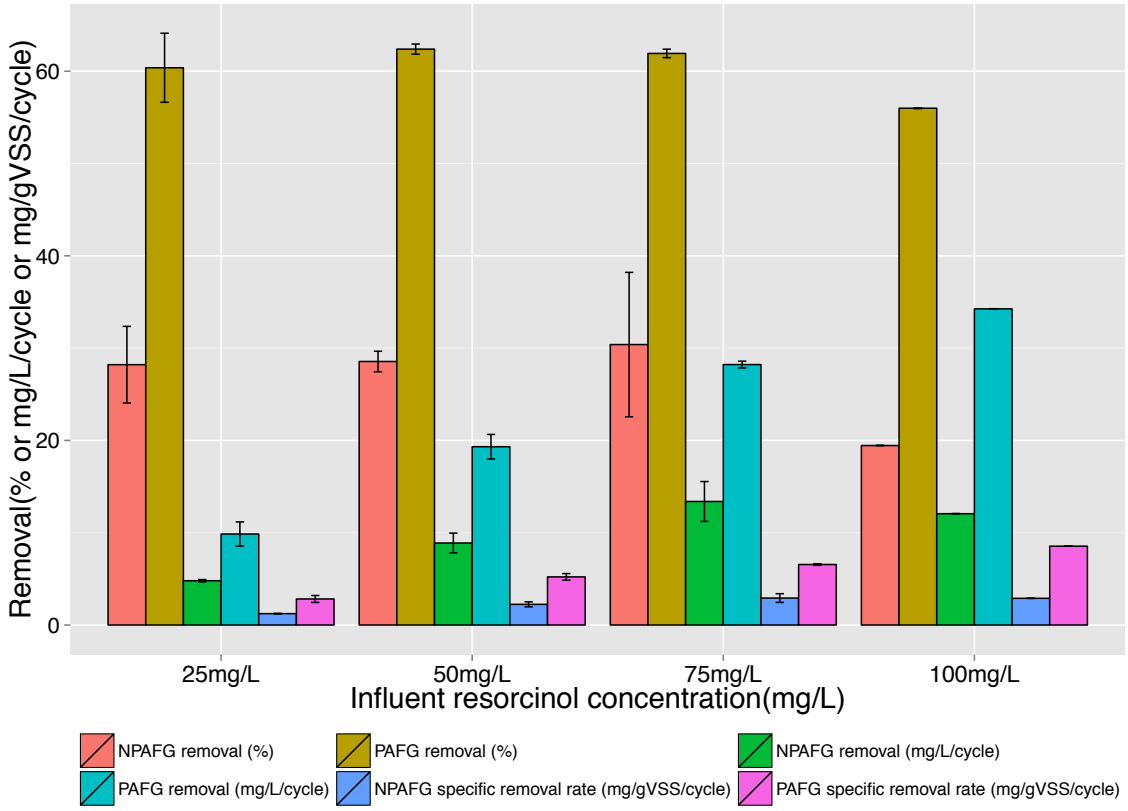



**Figure 23.** Final removal and specific removal rates of resorcinol at four different influent concentrations. Every bar represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

**Table 7.** Two-sample t-test results of resorcinol removal between PAFG reactor and NPAFG reactor. Numbers are averages and spread of duplicate tests.

| Resorcinol concentration in feed (mg/L) | Resorcinol removal percentages in PAFG | Resorcinol removal percentages in NPAFG | P-value for resorcinol removal percentages |
|---|---|---|---|
| 25 | 60.37 ± 5.29 | 28.20 ± 5.87 | **0.0289** |
| 50 | 62.40 ± 0.78 | 28.55 ± 1.57 | **0.0013** |
| 75 | 61.93 ± 0.65 | 30.38 ± 11.06 | 0.0565 |
| 100 | 55.99 ± 0.07 | 19.45 ± 0.05 | **2.7037E-06** |

From Figure 24, the PAFG reactor had soluble COD removal percentages greater than 80% during one cycle of treating resorcinol-containing synthetic wastewater, while the NPAFG

reactor had soluble COD removal percentages around 70%. The p-values of the t-tests for soluble COD removal were smaller than critical p-value 0.05, except for with the 25 mg/L resorcinol concentration (Table 8). The PAFG reactor had higher soluble COD removal percentages by 10 percentage points (80% in the PAFG versus 70% in the NPAFG). The existence of resorcinol also influenced the COD performance of the NPAFG reactor. From Figure 24, the soluble COD removal trend was similar to the catechol removal trend and the NPAFG reactor had plateaus for the soluble COD removal under higher resorcinol concentrations.

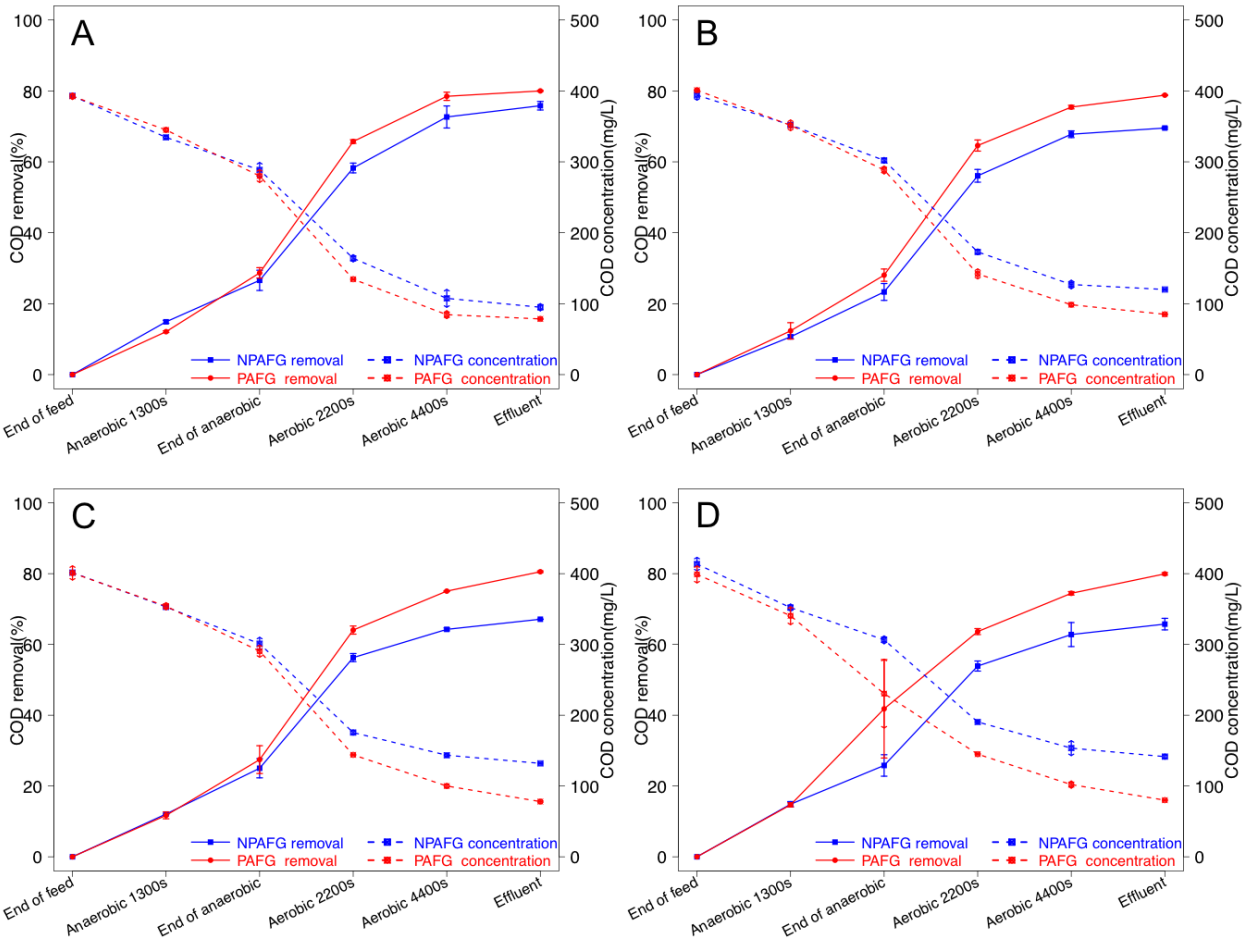



**Figure 24.** The soluble COD removals corresponding to the resorcinol test in Figure 22 under four different resorcinol concentrations: (A) 25 mg/L, (B) 50 mg/L, (C) 75 mg/L and (D) 100 mg/L (after the

phenol-enrichment under the corresponding concentration). The influent soluble COD concentration was 600 mg/L. Every point represents the average value of two duplicate experiments done successively with at least a two-cycle (1 HRT) interval. The error bars represent actual values obtained in the two duplicate experiments. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

Since one of the phenol degradation pathways and resorcinol pathway share a common enzyme, phenol 2-monooxygenase, phenol-enrichment likely boosted the microbes that can also degrade resorcinol. And because resorcinol has low biodegradability[74], it is not surprising that the NPAFG reactor had a poor removal performance even at 25 mg/L resorcinol concentration. Even after the phenol-enrichment, the performance of the PAFG reactor was still not that good compared to hydroquinone and catechol removal performances. It can be concluded that the phenol-enrichment process did enrich the microbes that can degrade resorcinol, but resorcinol removal was the worst of all the dihydroxybenzenes.

**Table 8.** Two-sample t-test results of soluble COD removal for resorcinol tests between PAFG reactor and NPAFG reactor. Numbers are averages and spread of duplicate tests.

| Resorcinol concentration in feed (mg/L) | Soluble COD removal percentages in PAFG | Soluble COD removal percentages in NPAFG | P-value for soluble COD removal percentages |
|---|---|---|---|
| 25 | $80.00 \pm 0.22$ | $75.83 \pm 1.71$ | 0.076 |
| 50 | $78.80 \pm 0.20$ | $69.54 \pm 0.41$ | **0.0012** |
| 75 | $80.54 \pm 0.33$ | $67.12 \pm 0.06$ | **0.0003** |
| 100 | $79.94 \pm 0.50$ | $65.74 \pm 2.31$ | **0.0136** |

### 4.3.4 Mixture of four compounds removal test results

After the PAFG reactor had a good performance on 100 mg/L phenol influent concentration, a mixture test was conducted.

In Figure 25 and 26, it is shown that the PAFG reactor displayed good removal of all of the four compounds, and the removal percentages for all the four compounds were around 80% (range from 79% to 86%). However, the NPAFG reactor had a worse performance compared to the PAFG reactor. For the NPAFG reactor the removal percentages of catechol and phenol were all very low, around 30%. Even for the most readily degradable dihydroxybenzene, hydroquinone, the removal was only around 55% in comparison to 86% in the PAFG. Phenol-enrichment improved the removal performances of the PAFG reactor on all of the three dihydroxybenzenes removal. Interestingly, among the four compounds, phenol and catechol had the lowest removal percentage in both of the reactors when the mixture was provided, while resorcinol had a higher removal percentage than when it was provided individually. This may be partly explained by the overlap of the phenol and catechol degradation pathways, but it is unclear why resorcinol removal improved compared to experiments when it was added individually. Phenol and catechol degradation rate decreased when they existed at the same time. As shown in the Figure 25, there were parallel concentration trends for all the four compounds in the PAFG reactor once the aerobic phase started, but in the anaerobic phase the dihydroxybenzenes' concentrations decreased more rapidly than phenol concentrations – possible due to differences in sorption onto biomass or to biochemical reaction differences such as preferential substrates. For the NPAFG reactor, slow and steady decreases of concentrations were observed for all compounds with both sorption and biochemical conversion likely contributing to removal.

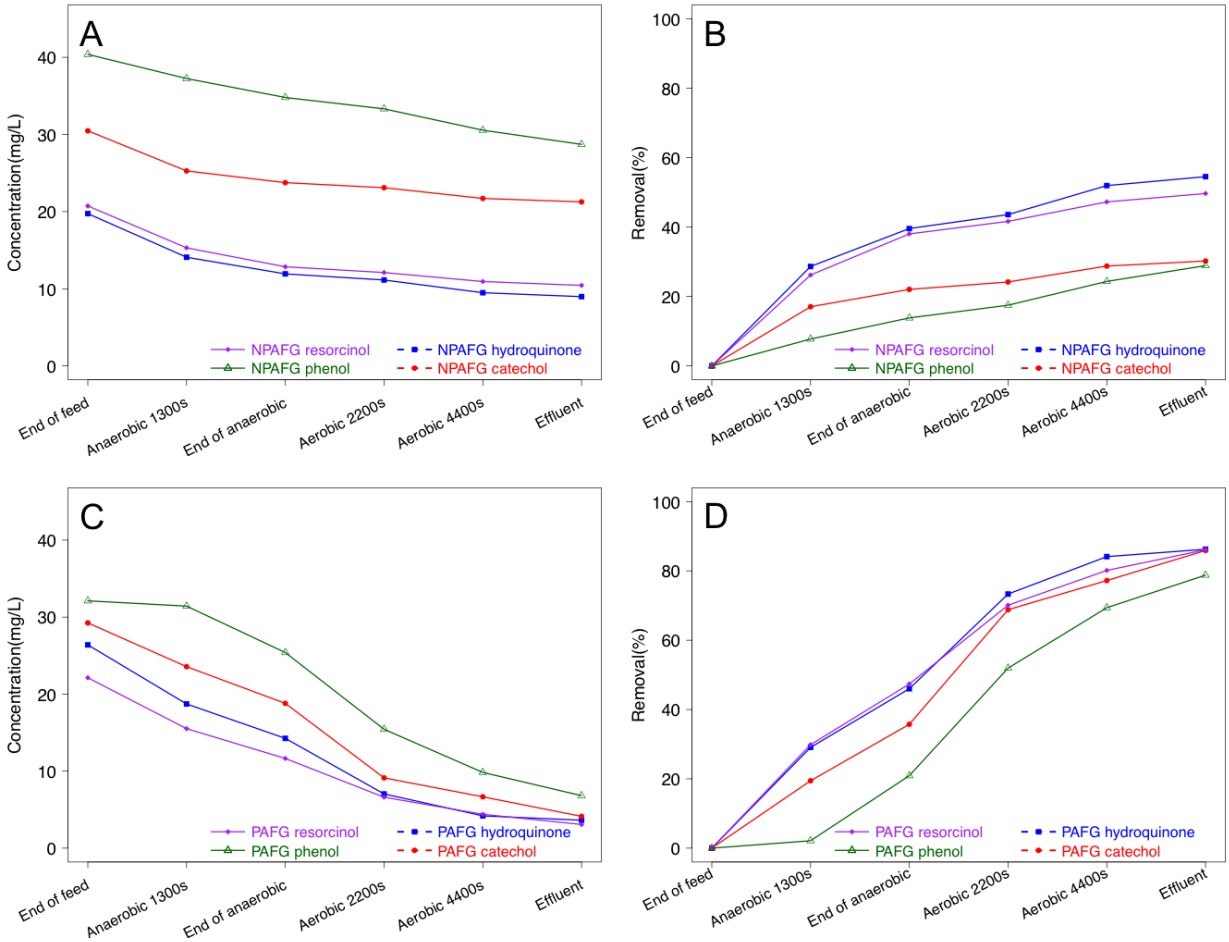



**Figure 25.** Removal of hydroquinone, catechol, phenol and resorcinol in a mixture of 50 mg/L each (after the phenol-enrichment under 100 mg/L concentration) in (A, B) NPAFG and (C, D) PAFG reactor. The left panel shows concentration over a cycle and right panel shows removal percentage over a cycle. The total influent soluble COD was 600 mg/L. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

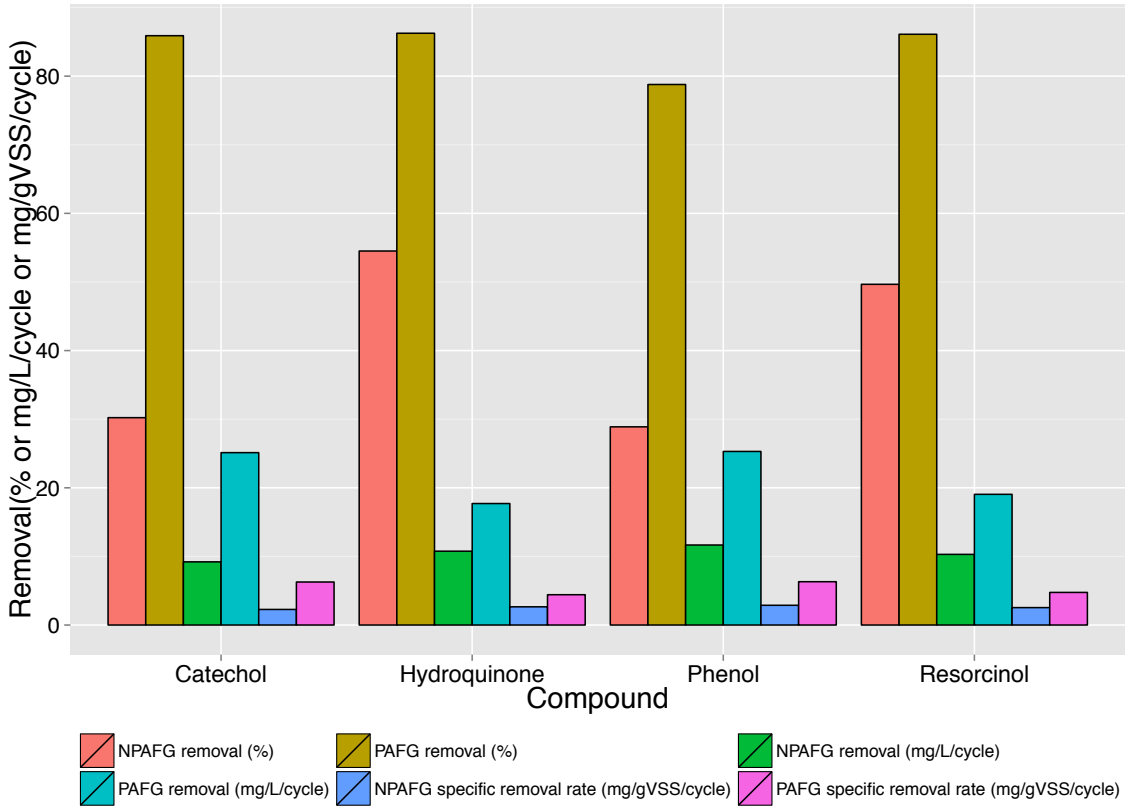



**Figure 26.** Final removal and specific removal rates of four compounds in a mixture of 50 mg/L each. Only one replicate of this experiment was performed. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

The PAFG reactor (Figure 27) had a soluble COD removal percentage around 80% during one cycle of treating the mixture synthetic wastewater, while the NPAFG reactor had a soluble COD removal percentage around 55%. The coexistence of the four compounds therefore influenced the COD removal performance of the NPAFG reactor. It is not surprising given that the aromatic compound comprised 68% of the added soluble COD in this experiment. From Figure 27, the rate of soluble COD removal increased as aerobic phase started, but the rate slowed with time[55,67,72].

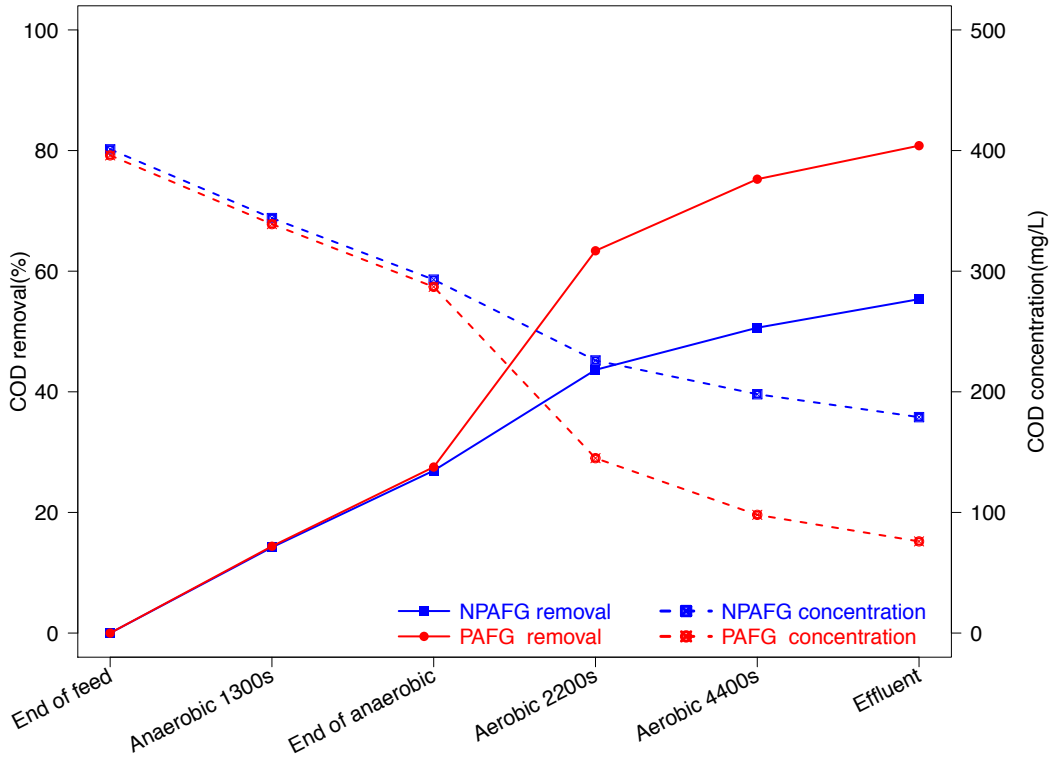



**Figure 27.** The soluble COD removal corresponding to the mixture test in Figure 25 in a mixture of hydroquinone, catechol, phenol and resorcinol at 50mg/L each (after the phenol-enrichment under 100 mg/L concentration). The total influent soluble COD was 600 mg/L. NPAFG: non-phenol-enriched acetate-fed granules. PAFG: phenol-enriched acetate-fed granules.

From the mixture test, it can be concluded that the phenol-enrichment did increase the degradation of hydroquinone, catechol and resorcinol.

### 4.3.5 Kinetics of removal tests

Table 9 shows removal rates of all chemicals during various times of a single cycle calculated from the above tests. As shown in Table 9, for phenol there was no obvious trend for anaerobic specific removal rates with the increase of phenol concentration. For phenol during the aerobic phase, the specific removal rates, except for 25 mg/L, were largest at the start of the aerobic

phase, 5 - 8 mg phenol · g $VSS^{-1}$ · $h^{-1}$, and decreased with time, 2 mg phenol · g $VSS^{-1}$ · $h^{-1}$ at the end. Using Haldane parameters reported by Tay and colleagues (2004) for aerobic granular sludge being fed phenol as the exclusive carbon source (parameters: $V_{max} = 1.7 \pm 0.2$ g phenol g $VSS^{-1}$ $d^{-1}$ $K_s = 40.3 \pm 3.1$ mg $L^{-1}$, and $K_i = 980.7 \pm 56.6$ mg $L^{-1}$) specific phenol degradation rates could be predicted between phenol concentrations from 0 to 200 mg phenol $L^{-1}$.[86] (Note that all phenol concentrations studied in this thesis had concentrations (up to 50 mg $L^{-1}$) that were well below the $K_i$ value (981 mg $L^{-1}$), so the Haldane equation essentially collapses to a simple Monod model). However, the actual specific phenol removal rates at the beginning of the aerobic phase in this thesis (25 mg/L: 0 mg phenol · g $VSS^{-1}$ · $h^{-1}$ , 50 mg/L: 4.9 mg phenol · g $VSS^{-1}$ · $h^{-1}$, 75 mg/L: 5.8 mg phenol · g $VSS^{-1}$ · $h^{-1}$, 100 mg/L: 8.2 mg phenol · g $VSS^{-1}$ · $h^{-1}$) were lower than the values predicted by the Haldane equation with the literature-reported parameter values (25 mg/L: 14.6 mg phenol · g $VSS^{-1}$ · $h^{-1}$ , 50 mg/L: 29.4 mg phenol · g $VSS^{-1}$ · $h^{-1}$, 75 mg/L: 36.1 mg phenol · g $VSS^{-1}$ · $h^{-1}$, 100 mg/L: 40.7 mg phenol · g $VSS^{-1}$ · $h^{-1}$). The actual rates normalized to the rates predicted by the equation of Tay et al. were 0.167, 0.160, and 0.201 for the 50 mg/L, 75 mg/L and 100 mg/L phenol levels, respectively. In the current study, the phenol-degrader community was only a part of the whole community. If we compare relative specific rates between the Tay et al. study and this research, they reflect the fraction of phenol in the feed media. In the Tay et al. 2004 publication, the whole population relied on phenol (directly or indirectly) for organic carbon. In this study that is not the case. For example, at the 50 mg phenol/L level, phenol comprises ~20% (0.2 fractionally) of the influent COD with the balance as acetate. Additionally, the different biomasses have different fractions of the VSS that is active biomass. Without SRT values for the current study system, direct comparison with past studies is difficult.

For hydroquinone, during the anaerobic phase the specific removal rates of the PAFG reactor (2 - 6.5 mg hydroquinone · g $VSS^{-1}$ · $h^{-1}$) were higher than those of the NPAFG reactor (1.5 - 3 mg hydroquinone · g $VSS^{-1}$ · $h^{-1}$) (Table 9). The anaerobic removal may be caused by biotransformation by phenol-enriched biomass or sorption of hydroquinone during the spike-in tests. During the aerobic phase, for both of the reactors, the specific removal rates were largest at the start of aerobic phase, 2 - 13 mg hydroquinone · g $VSS^{-1}$ · $h^{-1}$ in the PAFG reactor and 1.5 - 9.5 mg hydroquinone · g $VSS^{-1}$ · $h^{-1}$ in the NPAFG reactor, and decreased with time, 0.25 - 1 mg hydroquinone · g $VSS^{-1}$ · $h^{-1}$ in the PAFG reactor and 0.3 - 1 mg hydroquinone · g $VSS^{-1}$ · $h^{-1}$ in the NPAFG reactor at the end[55,67,72]. The specific removal rates at the beginning of the aerobic phase under four concentrations in the PAFG reactor were larger than those in the NPAFG reactor, while the specific removal rates at the middle and end of the aerobic phase for the two reactors were indistinguishable.

For catechol, during the anaerobic phase the specific removal rates of the PAFG reactor (2 - 5.5 mg catechol · g $VSS^{-1}$ · $h^{-1}$) were slightly higher than that of the NPAFG reactor (2 - 3.7 mg catechol · g $VSS^{-1}$ · $h^{-1}$) (Table 9). During the aerobic phase, for both of the reactors, the specific removal rates were largest at the start of aerobic phase, 3 - 11 mg catechol · g $VSS^{-1}$ · $h^{-1}$ in the PAFG reactor and 2 - 5 mg catechol · g $VSS^{-1}$ · $h^{-1}$ in the NPAFG reactor, and decreased with time, 0.05 - 1.5 mg catechol · g $VSS^{-1}$ · $h^{-1}$ in the PAFG reactor and 0 - 1 mg catechol · g $VSS^{-1}$ · $h^{-1}$ in the NPAFG reactor at the end. The specific removal rates at the start of the aerobic phase under four concentrations in the PAFG reactor were larger than those in the NPAFG reactor. Especially with influent concentration of 75 mg/L and 100 mg/L, the specific removal

rates at the beginning of the aerobic phase in the PAFG reactor (6.4 and 10.8 mg catechol · g $VSS^{-1}$ · $h^{-1}$) were double of those in the NPAFG reactor in the same time period (2.8 and 5 mg catechol · g $VSS^{-1}$ · $h^{-1}$). While the specific removal rates at the middle and end of the aerobic phase for the two reactors were indistinguishable.

For resorcinol, during the anaerobic phase the specific removal rates of the PAFG reactor (1.5 - 3.7 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$) were higher than those of the NPAFG reactor (1.1 - 2.3 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$). During the aerobic phase, for both of the reactors, the specific removal rates were not largest at the beginning of the aerobic phase, which was a feature of catechol and hydroquinone degradation patterns. The aerobic specific removal rates in both reactors maintained at the same level from the beginning to the end of the aerobic phase[55,67,72]. The aerobic specific removal rates in the PAFG reactor (0.9 - 3.5 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$) increased with the increase of the resorcinol concentration, while in the NPAFG reactor the aerobic specific removal rates (average around 0.6 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$) stayed the same with the increase of the resorcinol concentration. The aerobic specific removal rates in the PAFG reactor (from 0.9 to 3.5 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$) were more than double of those in the NPAFG reactor (from 0.4 to 1.5 mg resorcinol· g $VSS^{-1}$ · $h^{-1}$).

Among the three dihydroxybenzenes, in the PAFG reactor the anaerobic specific removal rates for hydroquinone and catechol were at the same level (2 - 6 mg · g $VSS^{-1}$ · $h^{-1}$), while the anaerobic specific removal rates for resorcinol were lower (1.5 - 4 mg resorcinol· g $VSS^{-1}$ · $h^{-1}$). During the aerobic phase, in the PAFG reactor the aerobic specific removal rates for hydroquinone and catechol were at the same level (2 - 13 mg hydroquinone· g $VSS^{-1}$ · $h^{-1}$ and

2.6 - 11 mg catechol · g $VSS^{-1}$ · $h^{-1}$ at the beginning of the aerobic phase, 0.1 - 2.5 mg hydroquinone· g $VSS^{-1}$ · $h^{-1}$ and 0 - 1.5 mg catechol· g $VSS^{-1}$ · $h^{-1}$ at the middle of the aerobic phase and 0.2 - 1 mg hydroquinone· g $VSS^{-1}$ · $h^{-1}$ and 0 - 1 mg catechol· g $VSS^{-1}$ · $h^{-1}$ at the end of the aerobic phase), while the aerobic specific removal rates for resorcinol at the beginning of the aerobic phase were lower than those of hydroquinone and catechol and higher than those of hydroquinone and catechol at the end of the aerobic phase (1 - 5 mg resorcinol· g $VSS^{-1}$ · $h^{-1}$ at the beginning of the aerobic phase, 0.8 - 2 mg resorcinol· g $VSS^{-1}$ · $h^{-1}$ at the middle of the aerobic phase and 0.9 - 5 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$ at the end of the aerobic phase).

In the NPAFG reactor the anaerobic specific removal rates for the three dihydroxybenzenes were similar (1 - 3 mg · g $VSS^{-1}$ · $h^{-1}$) and during the aerobic phase, in the NPAFG reactor the aerobic specific removal rates at the beginning of the aerobic phase from high to low were hydroquinone (1.5 - 10 mg hydroquinone· g $VSS^{-1}$ · $h^{-1}$), catechol (2 - 5 mg catechol· g $VSS^{-1}$ · $h^{-1}$) and resorcinol (0.3 - 0.8 mg resorcinol · g $VSS^{-1}$ · $h^{-1}$). At the middle and the end of the aerobic phase, in the NPAFG reactor the aerobic specific removal rates for the three dihydroxybenzenes were at the same level (average 1 mg · g $VSS^{-1}$ · $h^{-1}$ at the middle of the aerobic phase and average 0.5 mg · g $VSS^{-1}$ · $h^{-1}$ at the end of the aerobic phase).

**Table 9.** Kinetics analysis for removal tests. *: negative values imply a slight decrease in measurements but do not imply production of the aromatic compounds. ND: not determined. Values are averages across duplicate experiments.

| | | Removal percentages (%) | | Aromatic compound removed (mg / L / cycle) | | Aromatic compound loading rate (mg / L / day) | Anaerobic specific removal rate (mg / g VSS /h) | | Aerobic specific removal rate (mg / g VSS / h) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Beginning | | Middle | | End | |
| **Compound** | **Influent concentration (mg / L)** | **PAFG** | **NPAFG** | **PAFG** | **NPAFG** | | **PAFG** | **NPAFG** | **PAFG** | **NPAFG** | **PAFG** | **NPAFG** | **PAFG** | **NPAFG** |
| **Phenol** | 25 | 90.47 | ND | 9.49 | ND | 100 | 2.44 | ND | -0.16* | ND | 1.61 | ND | 0 | ND |
| | 50 | 91.97 | ND | 26.93 | ND | 200 | 1.26 | ND | 4.85 | ND | 3.66 | ND | 1.83 | ND |
| | 75 | 90.27 | ND | 39.70 | ND | 300 | 1.47 | ND | 5.80 | ND | 5.01 | ND | 2.49 | ND |
| | 100 | 83.29 | ND | 49.22 | ND | 400 | 2.17 | ND | 8.17 | ND | 7.00 | ND | 2.26 | ND |
| **Hydroquinone** | 25 | 85.77 | 82.01 | 14.34 | 12.53 | 100 | 1.99 | 1.43 | 1.98 | 1.51 | 1.35 | 0.94 | 0.94 | 1.08 |
| | 50 | 92.52 | 85.50 | 25.56 | 19.34 | 200 | 6.44 | 2.82 | 2.83 | 2.30 | 0.08 | 1.67 | 0.48 | 0.55 |
| | 75 | 83.83 | 79.60 | 34.17 | 32.23 | 300 | 3.68 | 2.81 | 5.98 | 6.15 | 2.15 | 1.65 | 0.35 | 0.28 |
| | 100 | 87.95 | 81.14 | 54.78 | 41.98 | 400 | 5.54 | 2.74 | 12.84 | 9.53 | 2.47 | 2.54 | 0.24 | 1.05 |
| **Catechol** | 25 | 71.12 | 70.17 | 12.54 | 12.24 | 100 | 2.18 | 1.94 | 2.61 | 2.18 | 0.54 | 0.37 | 0.05 | 0.22 |
| | 50 | 80.20 | 64.92 | 24.05 | 20.76 | 200 | 3.86 | 3.39 | 3.64 | 2.18 | 1.39 | 1.17 | 0.86 | 1.05 |
| | 75 | 85.51 | 62.21 | 31.98 | 23.35 | 300 | 3.17 | 3.23 | 6.37 | 2.75 | 0.78 | 1.80 | 1.12 | -0.17* |
| | 100 | 80.99 | 55.62 | 46.79 | 29.50 | 400 | 5.62 | 3.74 | 10.77 | 5.03 | -0.07* | 2.24 | 1.50 | -0.28* |
| **Resorcinol** | 25 | 60.37 | 28.20 | 9.86 | 4.80 | 100 | 1.51 | 1.10 | 1.12 | 0.31 | 0.79 | 0.11 | 0.85 | 0.24 |
| | 50 | 62.39 | 28.55 | 19.32 | 8.89 | 200 | 1.99 | 1.54 | 2.04 | 0.77 | 1.91 | 0.32 | 2.14 | 0.69 |
| | 75 | 61.93 | 30.38 | 28.22 | 13.39 | 300 | 2.71 | 2.11 | 2.91 | 0.33 | 1.64 | 1.48 | 2.86 | 0.39 |
| | 100 | 55.99 | 19.45 | 34.25 | 12.06 | 400 | 3.70 | 2.33 | 3.81 | 0.82 | 0.76 | 0.59 | 4.87 | 0.46 |

# CHAPTER 5: CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDIES

Aerobic acetate-degrading granules were successfully cultivated within two months before phenol-enrichment. As shown in the soluble COD removal tests before phenol-enrichment, both the NPAFG and the PAFG reactors had good performance on acetate COD removal (> 90%) and they also showed significant nitrification (data not shown). Phenol-degrading granules haven't been cultivated rapidly from activated sludge directly so acetate-fed granules were used as microbial seed to get phenol-degrading granular sludge[40].

For hydroquinone, because it can be readily biodegraded by various groups of microorganisms[60,84,87] and granules have a diverse microbial community[46], even without phenol-enrichment the NPAFG reactor had 80% removal percentage for hydroquinone (up to 42 mg hydroquinone $\cdot$ $L^{-1}$ $\cdot$ $cycle^{-1}$) and maintained its ability to remove > 75% soluble COD (including acetate). Although the differences between the final removal percentages of the PAFG and the NPAFG reactors were not large, the differences between them were statistically significant under higher hydroquinone concentrations (above 50 mg/L hydroquinone concentration). For catechol, with the increase of catechol concentration, the PAFG reactor maintained 80% catechol removal percentage, while the removal percentage of catechol dropped to 60% in NPAFG reactor. Both of the reactors had soluble COD removal percentages more than 80% during one 3-hour-cycle of treating catechol-containing synthetic wastewater, except for 100 mg/L catechol tests in the PAFG reactor showed around 8 percentage points higher soluble COD removal than NPAFG reactor. Since catechol is not as readily biodegradable as hydroquinone, the NPAFG reactor did

not have a good performance on catechol removal, especially under higher catechol concentrations. Because catechol is one of the intermediate products on one of the phenol degradation pathways, the phenol-enrichment process did enrich the microbes that can degrade catechol. The main aerobic phenol biodegradation pathway includes catechol, and enrichment of phenol degraders resulted in the improvement of catechol degradation.

For resorcinol, with the increase of resorcinol concentration the resorcinol removal percentage in the PAFG reactor remained around 60%, while the NPAFG reactor had removal percentages around 25%, and when the resorcinol concentration was increased to 100 mg/L, the NPAFG removal percentage decreased to 20%. Because resorcinol has a generally low rate of biodegradability[74], the NPAFG reactor had a poor removal performance even at 25 mg/L resorcinol concentration and even after the phenol-enrichment the performance of the PAFG reactor was still not that good compared to the results of hydroquinone and catechol. Since the phenol degradation pathway and resorcinol pathway share a common enzyme (phenol 2-monooxygenase), phenol-enrichment likely increased the percentage of the microbial population in the granules that can also degrade resorcinol.

Within a single cycle, the sharpest decreases in concentration occurred after the one-hour anaerobic phase, during the aerobic phase. This is not surprising given that most of the aromatic biodegradation pathways involve molecular oxygen as a substrate. Resorcinol was the one exception, whose removal rate during anaerobic phases was indistinguishable from rates during aerobic phases.

Regarding the removal percentage increase in the PAFG reactor versus the control reactor (NPAFG), phenol-enrichment boosted the resorcinol degradation most followed by catechol then hydroquinone. Because hydroquinone can be easily degraded by diverse organisms including those in acetate-fed granules, its degradation was improved least. Sorption is likely also contributing to some of the compound removals observed in this dataset.

For the single assay with mixtures of dihydroxybenzenes, the PAFG reactor had a good performance on all of the four compounds, and the removal percentage for all the four compounds were around 80%. However, in the NPAFG reactor the removal percentages of catechol and phenol were around 25% and hydroquinone and resorcinol were around 50%. This trending of phenol and catechol data may be caused by the overlap of the phenol and catechol degradation pathways, resulting in the interference between phenol and catechol degradations. Overall phenol-cultivation improved the performance of the PAFG reactor for dihydroxybenzenes removal.

This experiment suggests that enrichment with one aromatic compound can increase the degradation of structurally similar aromatic chemicals, which can be applied to GSBR applications. When a treatment plant periodically receives one or more aromatic compounds it could potentially use a similar strategy to the one used in this thesis research. As a preventive measure, the plant biomass can be fed with a benign (or efficiently degraded) structurally related compound to insure that the plant's biomass is always ready to receive and degrade the toxic compound(s). This could decrease the shock that the compounds would exert on the granular sludge if the concentrations in the influent changed suddenly. Thus granular GSBRs with phenol-

enriched biomass hold promise for successfully treating varying-quality wastewater containing the target group of structurally similar aromatic compounds. Logistically, to operate a GSBR with such a short feed time (10 min) for a 3-hour cycle would require either a large number of reactors operating together (180 min/10 min = 18 SBR reactors) or the presence of an influent holding tank if fewer reactors were to be used.

However, the genetic and molecular work is needed to reconfirm the mechanism behind the improvement seen in the PAFG reactor. Future researchers could trace the microbial community changes in the granules during the phenol-enrichment process to further explain the reason for the degradation improvement of the three dihydroxybenzenes. Proteomic and messenger RNA analyses would help prove that the enzymes produced along the phenol degradation pathways in the PAFG reactor (e.g. phenol 2-monooxygenase) assisted granules in degrading the three dihydroxybenzenes. Most importantly, to fully examine the kinetics and create models of biotransformation rates, more sampling points should be taken in one GSBR cycle, especially during the aerobic phase. Abiotic tests for sorption effects should also be run. Lower dissolved oxygen levels could be applied in order to achieve better energy efficiency and aeration time could be changed to find optimum aeration time for simultaneous COD removal, aromatics removal, nitrification and denitrification. To better monitor physical characteristics of sludge over time, Sludge Volume Index (SVI) could be measured in future studies and snapshots of granules could be taken along the research to quantitatively and qualitatively monitor the physical characteristics of granules. Effluent VSS and TSS should be measured to determine effluent quality with respect to meeting discharge limits and to evaluate solids retention time (SRT). Since resorcinol has low biodegradability[74], to achieve desired effluent levels other

wastewater treatment technologies might be coupled with GSBR to treat the resorcinol-containing wastewater.

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# APPENDIX

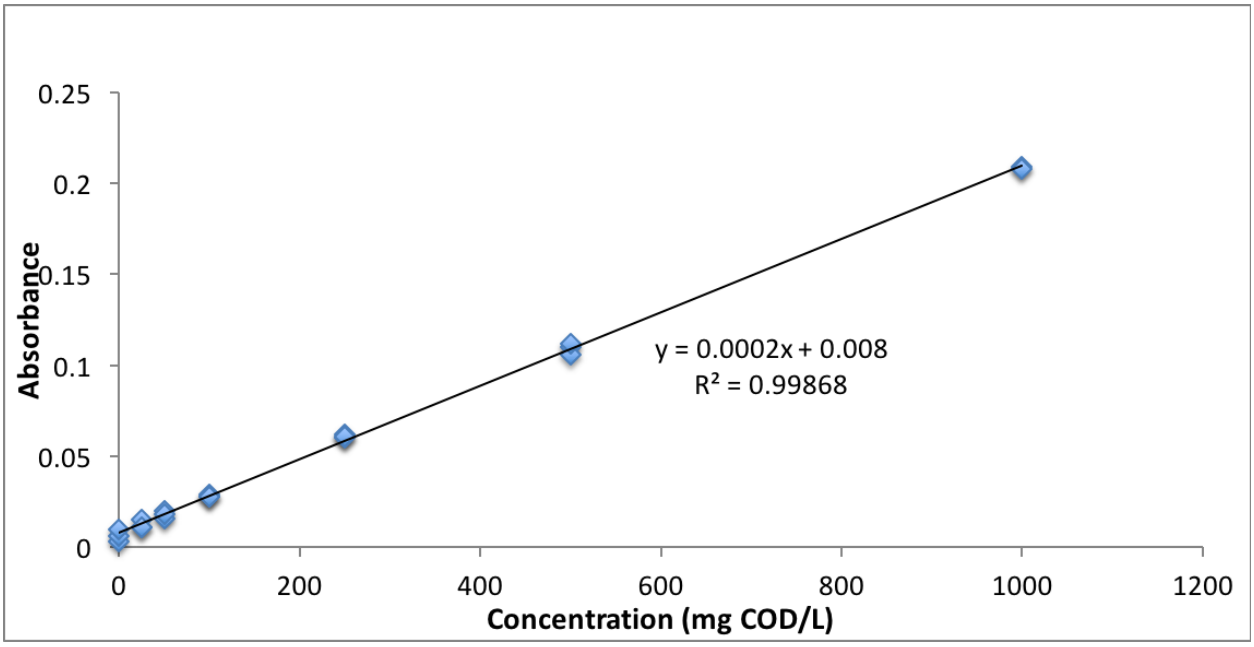


**Figure 28.** Standard curve for COD using NanoDrop under 620 nm wavelength.

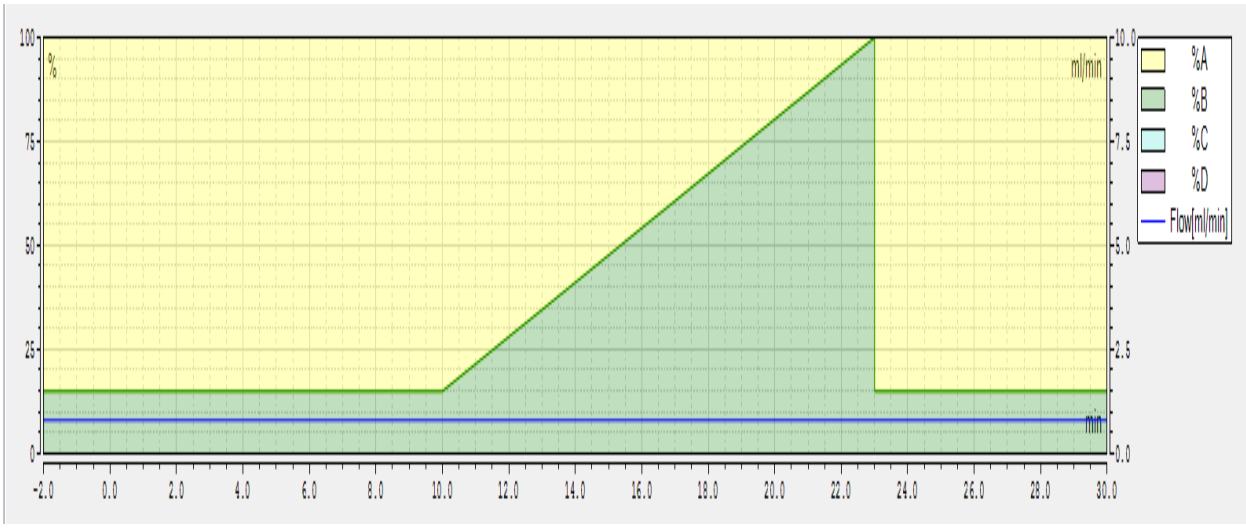


**Figure 29.** An eluent gradient mobile phase was applied to achieve separation of phenol, hydroquinone, catechol and resorcinol. The mobile phase consists of nanopure water (A) and HPLC-grade acetonitrile (B). The percentage of (A) was changed according to the program: 0 - 10 min, 15% B (isocratic); 10 - 23 min, 15 - 100% B (linear gradient); 23 - 30 min, 15% B (isocratic). The chromatographic conditions were room temperature; flow rate 0.8 mL/min.

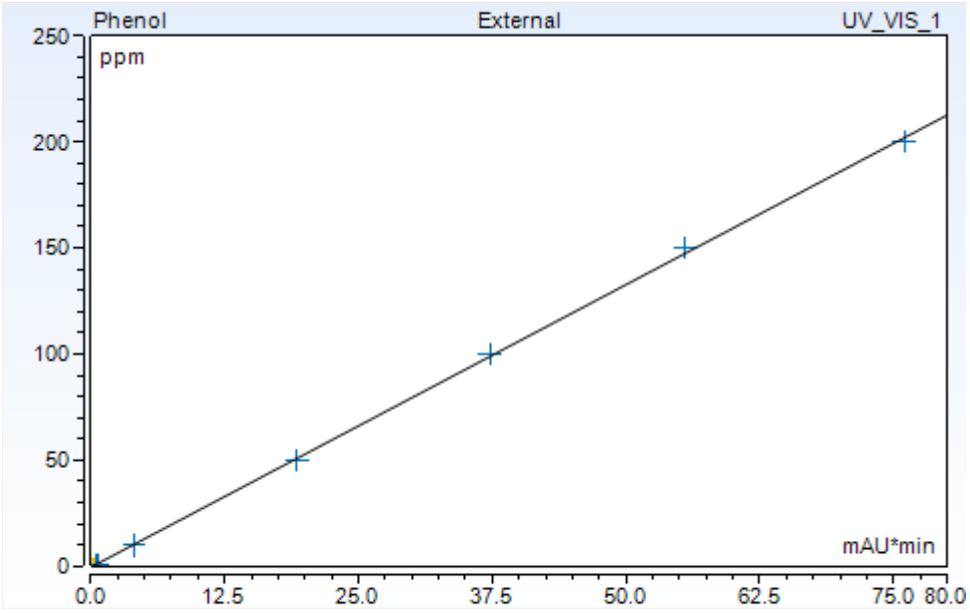


**Figure 30.** Calibration curve for phenol using HPLC-UV under 270nm. The coefficient of determination is 0.99972. The retention time for phenol is 19.2 min.

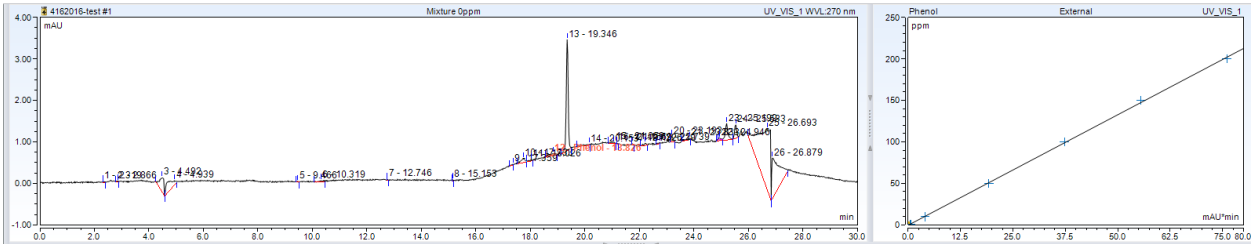


**Figure 31.** Pure water absorbance under 270 nm wavelength, which was used for phenol calibration.

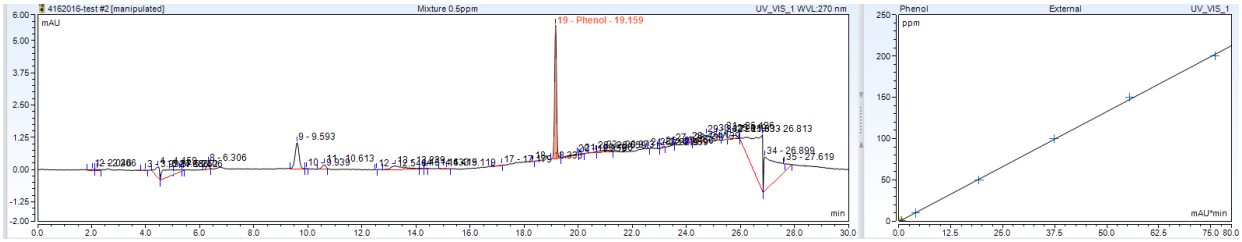


**Figure 32.** Mixture (phenol, hydroquinone, catechol and resorcinol at 0.5 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

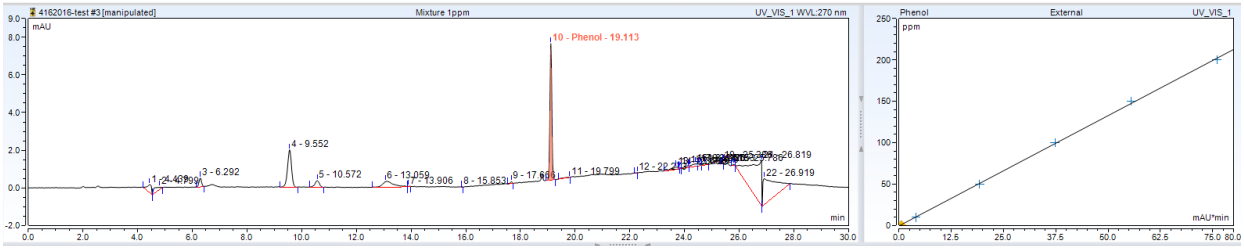

**Figure 33.** Mixture (phenol, hydroquinone, catechol and resorcinol at 1 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

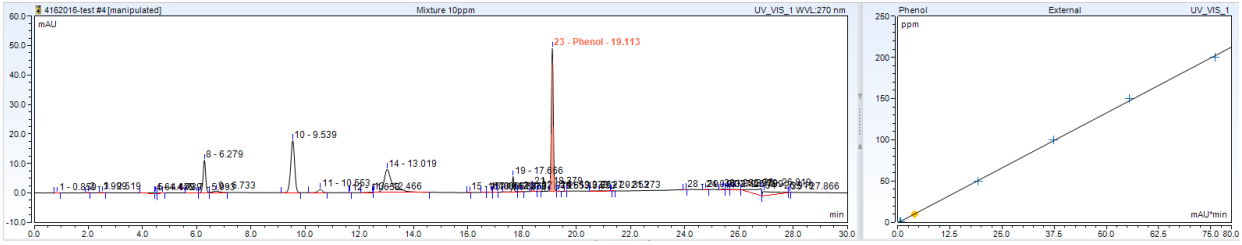


**Figure 34.** Mixture (phenol, hydroquinone, catechol and resorcinol at 10 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

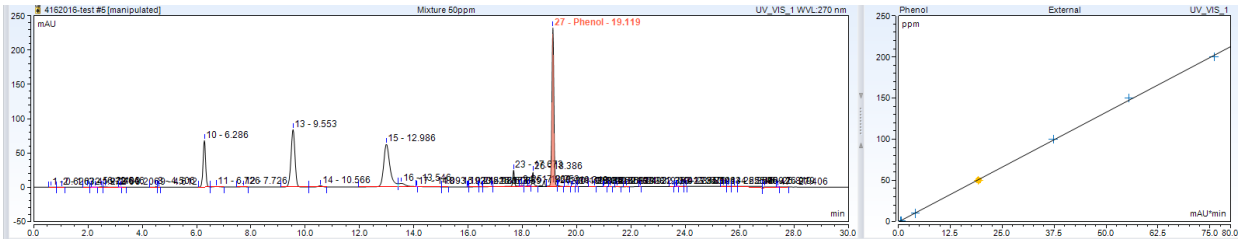


**Figure 35.** Mixture (phenol, hydroquinone, catechol and resorcinol at 50 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

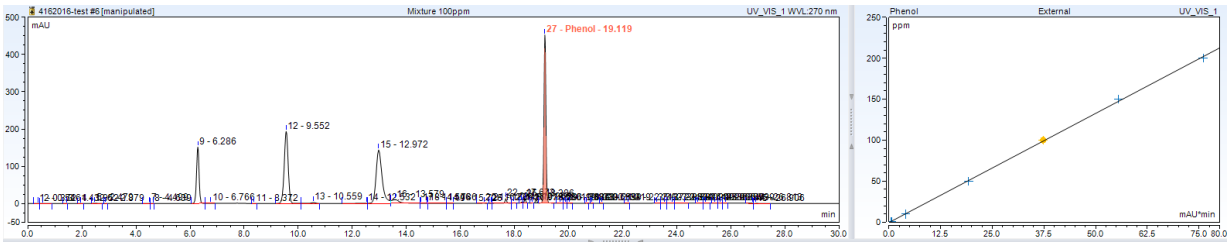


**Figure 36.** Mixture (phenol, hydroquinone, catechol and resorcinol at 100 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

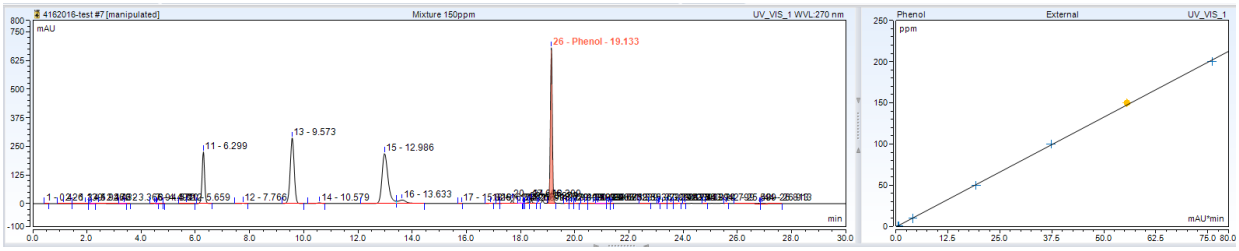


**Figure 37.** Mixture (phenol, hydroquinone, catechol and resorcinol at 150 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

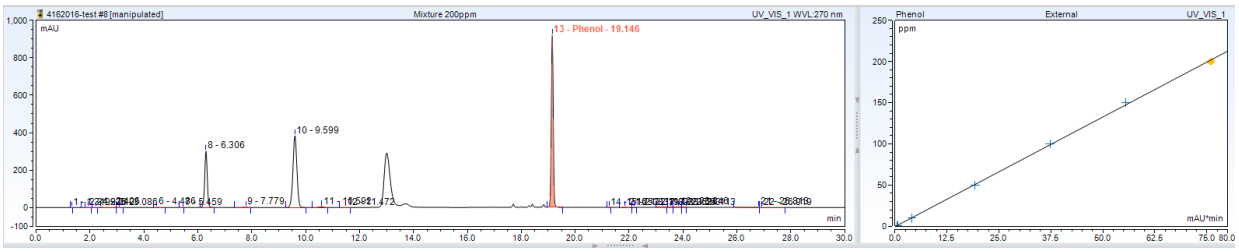


**Figure 38.** Mixture (phenol, hydroquinone, catechol and resorcinol at 200 mg/L each) absorbance under 270 nm wavelength, which was used for phenol calibration.

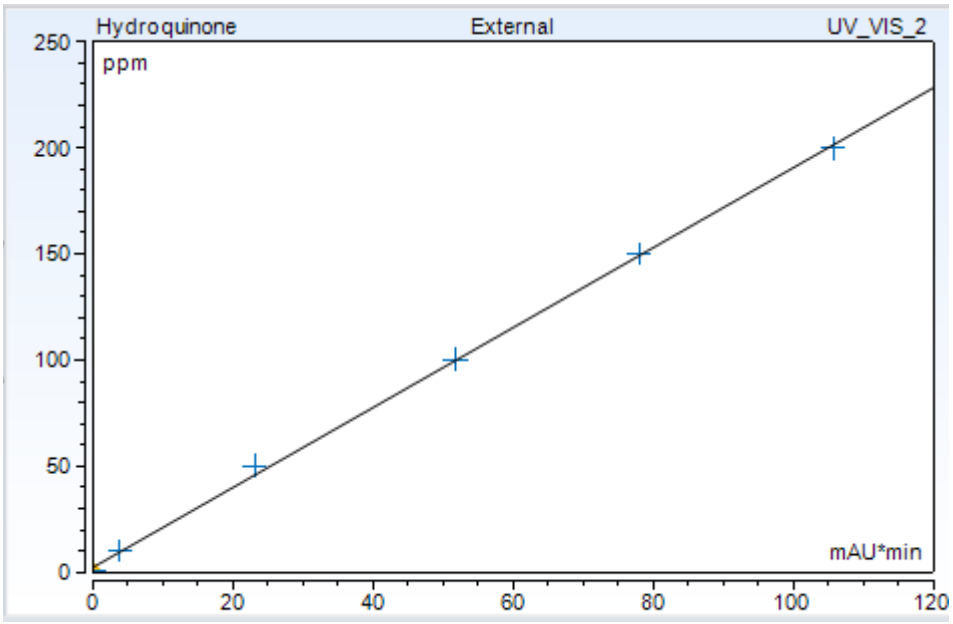


**Figure 39.** Calibration curve for hydroquinone using HPLC-UV under 292nm. The coefficient of determination is 0.99930. The retention time for phenol is 6.39 min.

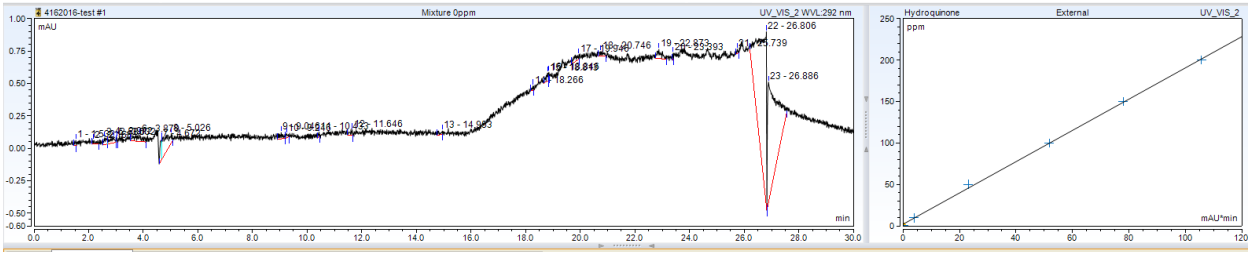


**Figure 40.** Pure water absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

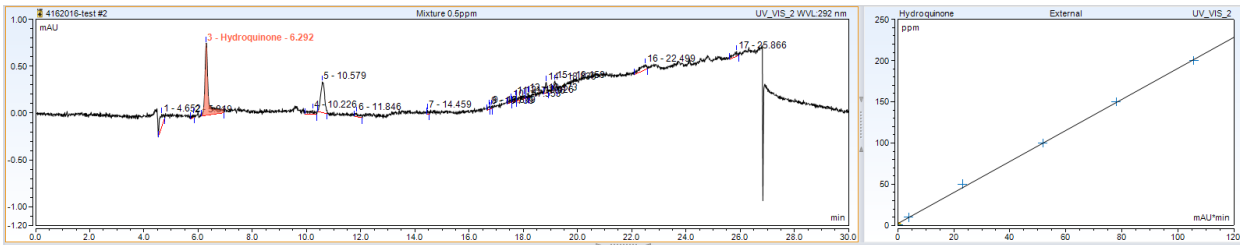


**Figure 41.** Mixture (phenol, hydroquinone, catechol and resorcinol at 0.5 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

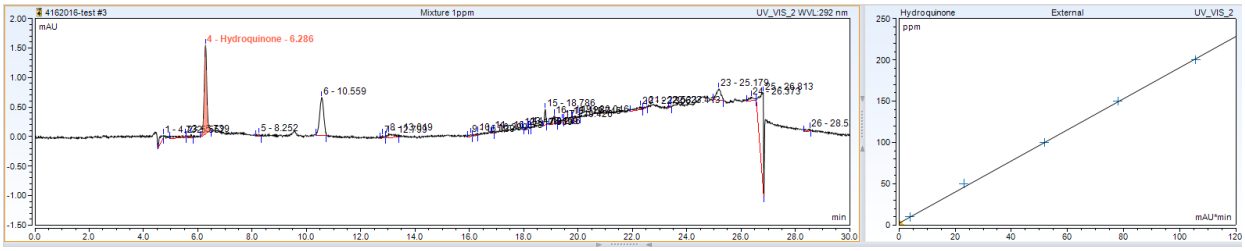


**Figure 42.** Mixture (phenol, hydroquinone, catechol and resorcinol at 1 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

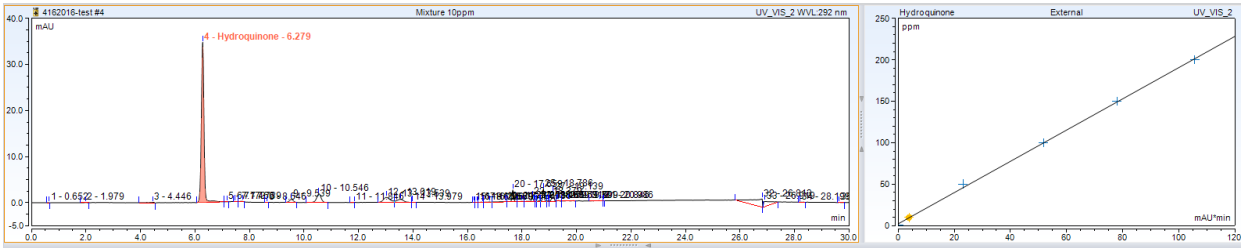


**Figure 43.** Mixture (phenol, hydroquinone, catechol and resorcinol at 10 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

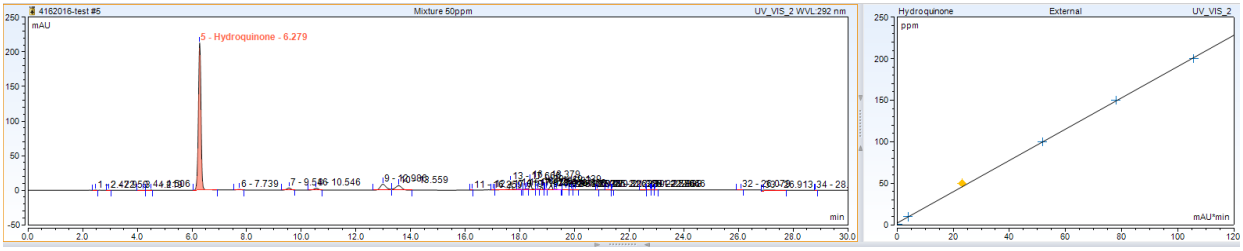


**Figure 44.** Mixture (phenol, hydroquinone, catechol and resorcinol at 50 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

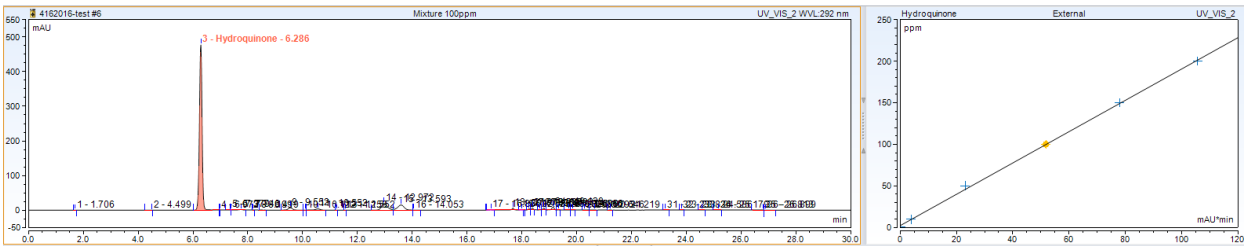


**Figure 45.** Mixture (phenol, hydroquinone, catechol and resorcinol at 100 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

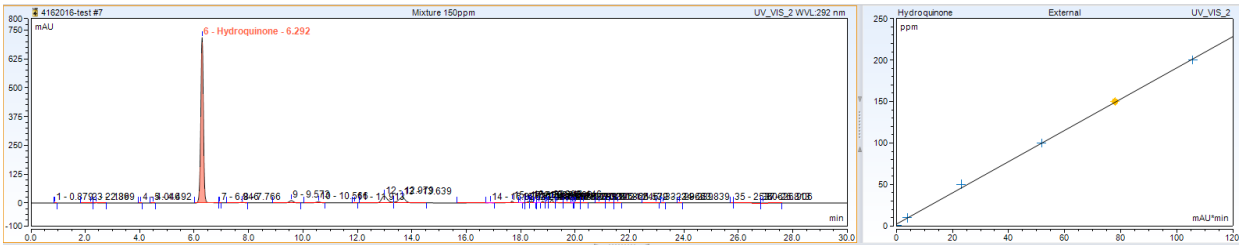


**Figure 46.** Mixture (phenol, hydroquinone, catechol and resorcinol at 150 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

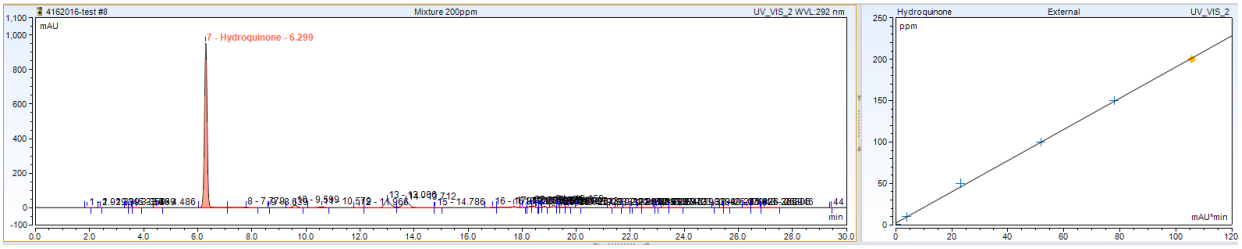


**Figure 47.** Mixture (phenol, hydroquinone, catechol and resorcinol at 200 mg/L each) absorbance under 292 nm wavelength, which was used for hydroquinone calibration.

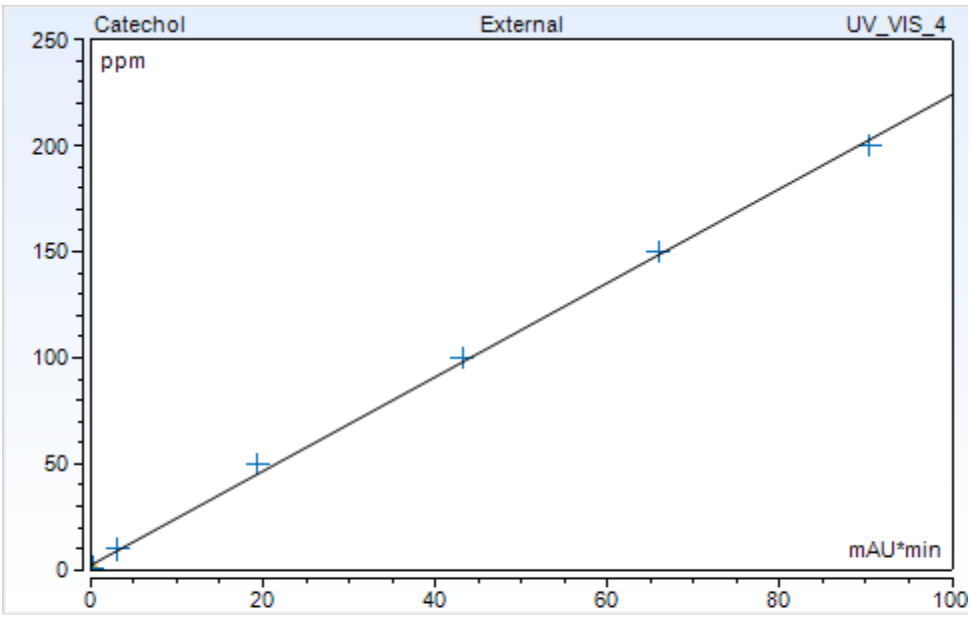


**Figure 48.** Calibration curve for catechol using HPLC-UV under 280nm. The coefficient of determination is 0.99882. The retention time for phenol is 13.4 min.

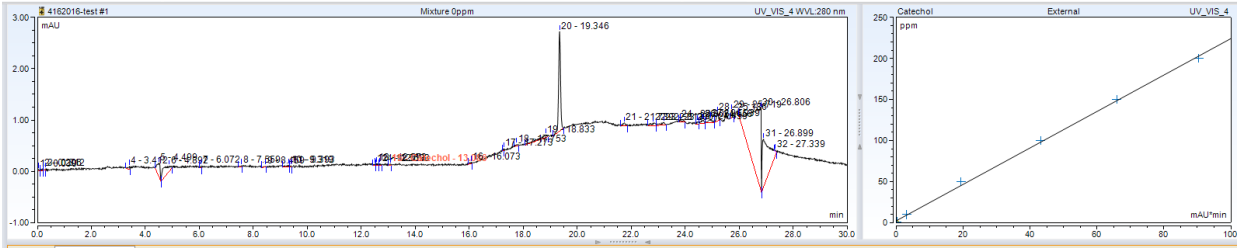

**Figure 49.** Pure water absorbance under 280 nm wavelength, which was used for catechol calibration.

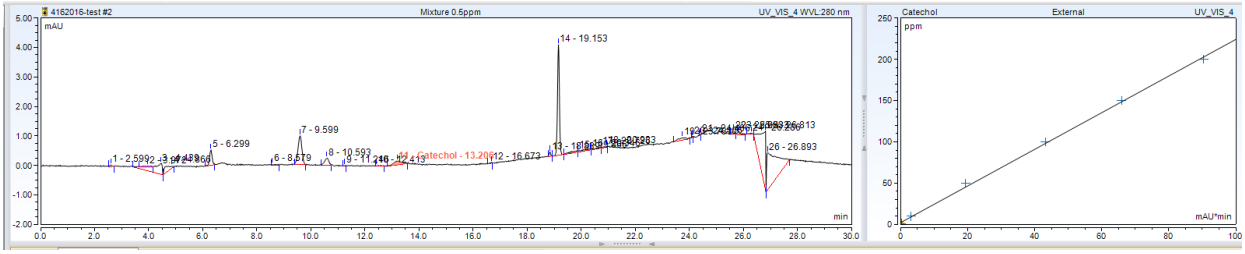

**Figure 50.** Mixture (phenol, hydroquinone, catechol and resorcinol at 0.5 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

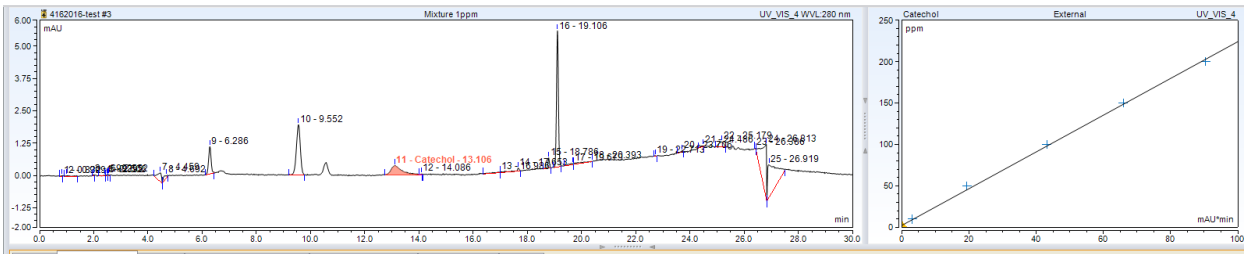


**Figure 51.** Mixture (phenol, hydroquinone, catechol and resorcinol at 1 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

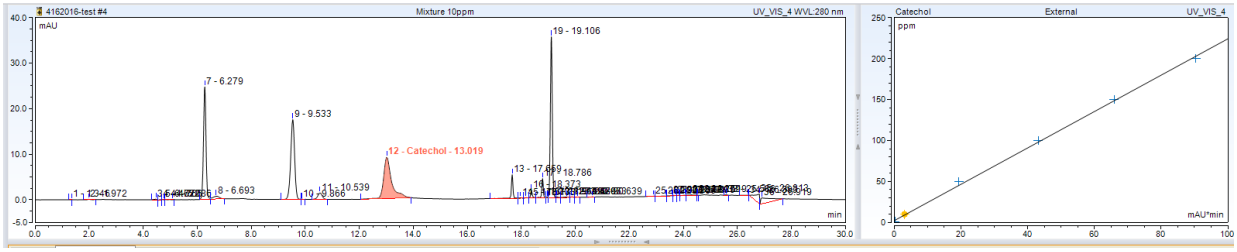


**Figure 52.** Mixture (phenol, hydroquinone, catechol and resorcinol at 20 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

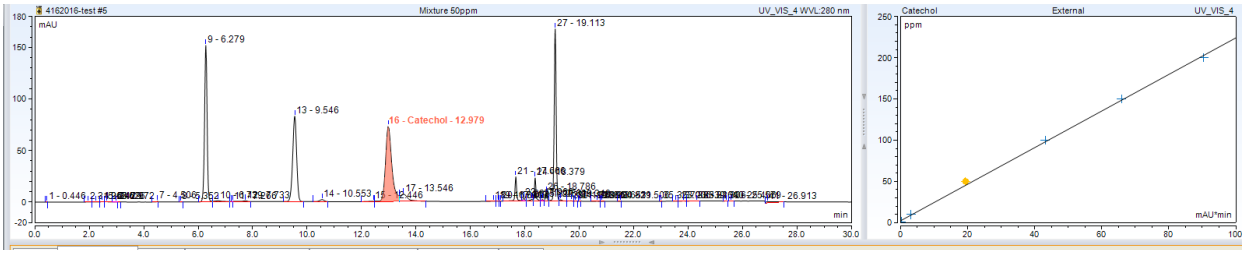


**Figure 53.** Mixture (phenol, hydroquinone, catechol and resorcinol at 50 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

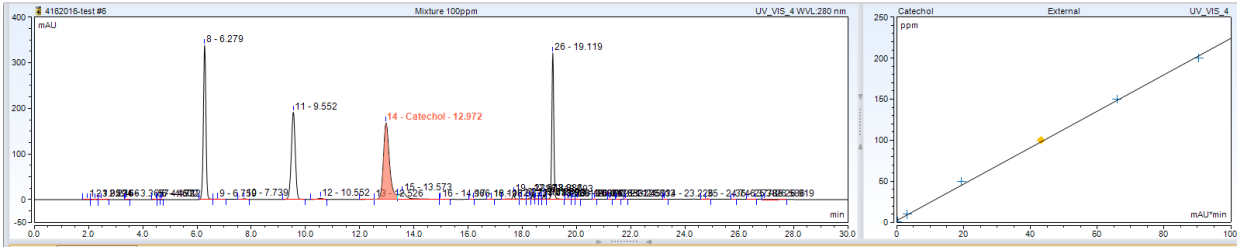


**Figure 54.** Mixture (phenol, hydroquinone, catechol and resorcinol at 100 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

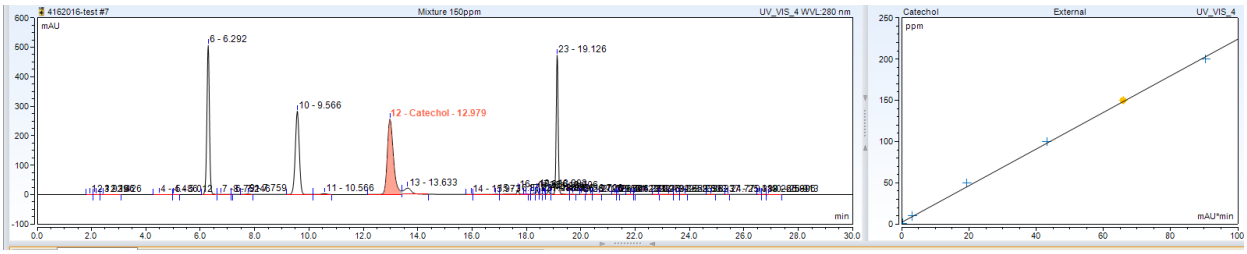


**Figure 55.** Mixture (phenol, hydroquinone, catechol and resorcinol at 150 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

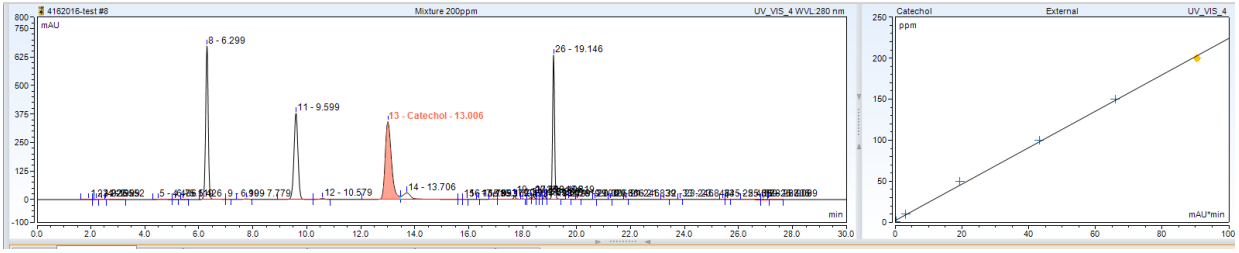


**Figure 56.** Mixture (phenol, hydroquinone, catechol and resorcinol at 200 mg/L each) absorbance under 280 nm wavelength, which was used for catechol calibration.

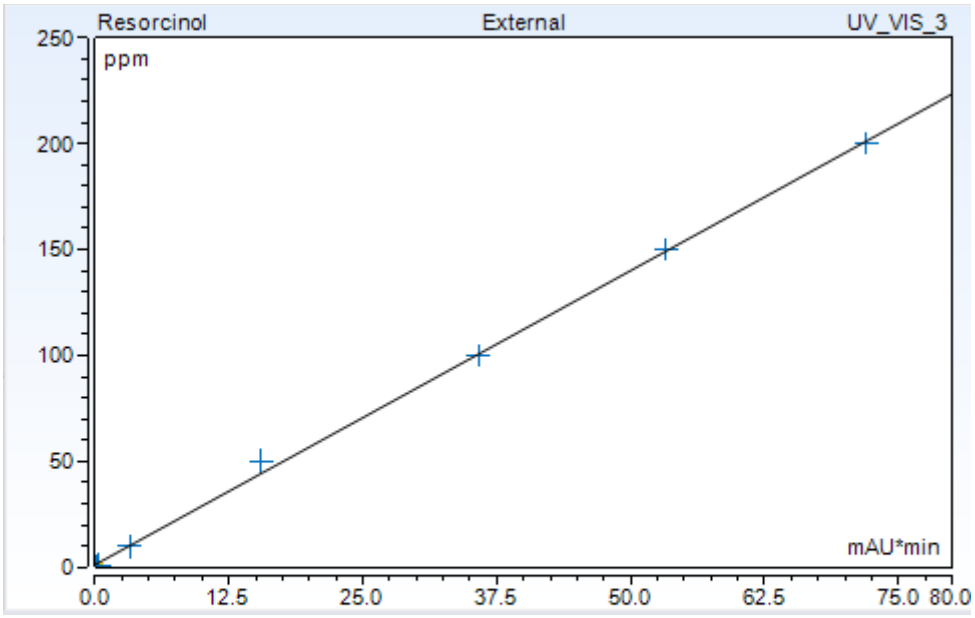


**Figure 57.** Calibration curve for resorcinol using HPLC-UV under 272 nm. The coefficient of determination is 0.99906. The retention time for phenol is 9.9min.

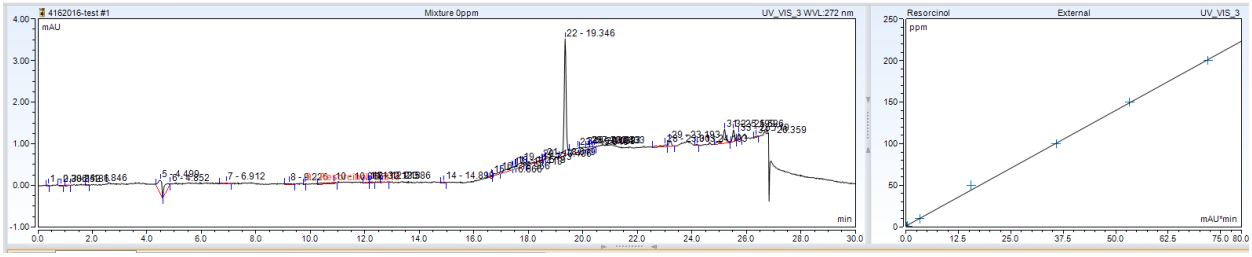


**Figure 58.** Pure water absorbance under 272 nm wavelength, which was used for resorcinol calibration.

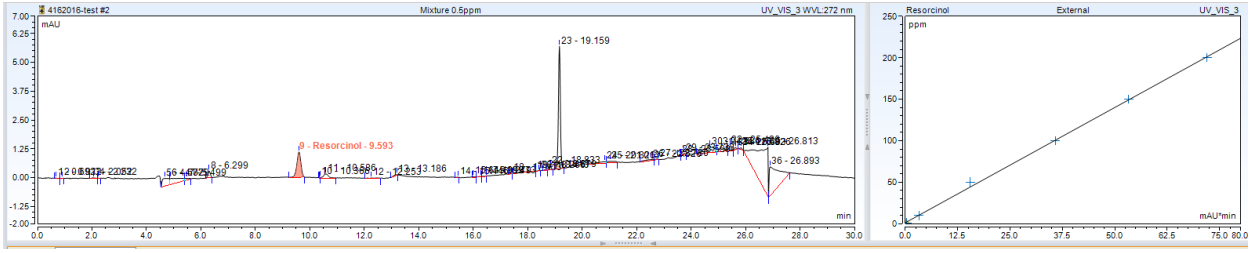


**Figure 59.** Mixture (phenol, hydroquinone, catechol and resorcinol at 0.5 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.

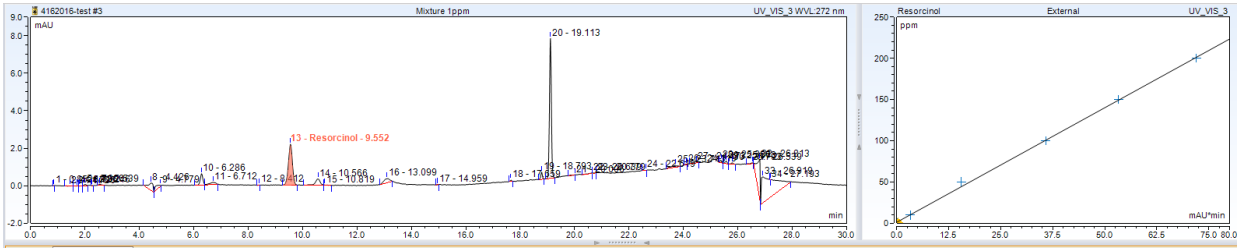


**Figure 60.** Mixture (phenol, hydroquinone, catechol and resorcinol at 1 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.

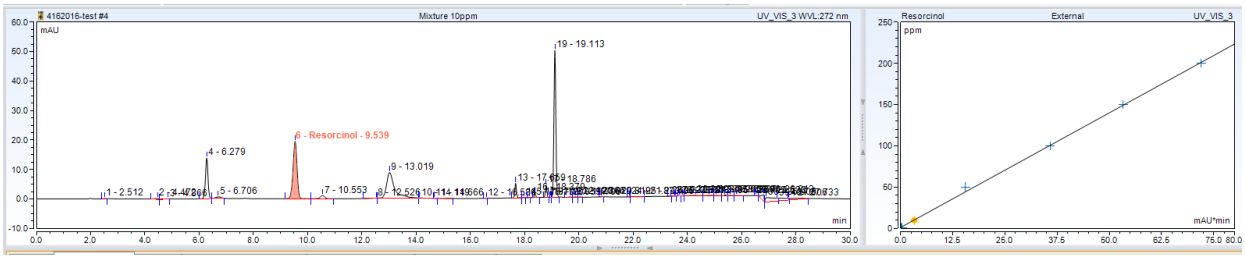


**Figure 61.** Mixture (phenol, hydroquinone, catechol and resorcinol at 10 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.

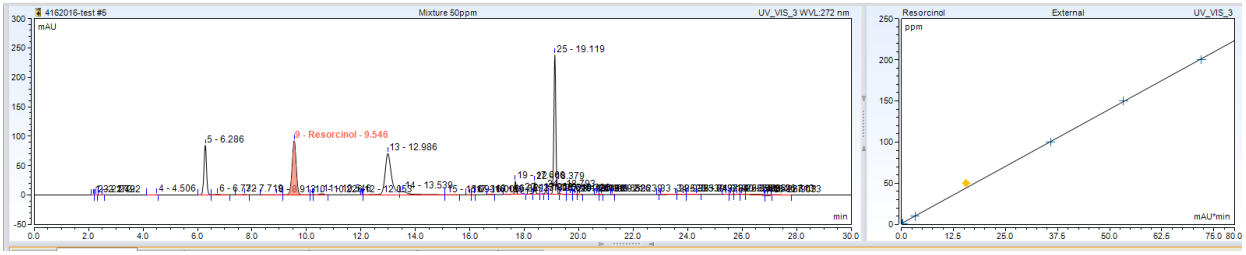


**Figure 62.** Mixture (phenol, hydroquinone, catechol and resorcinol at 50 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.

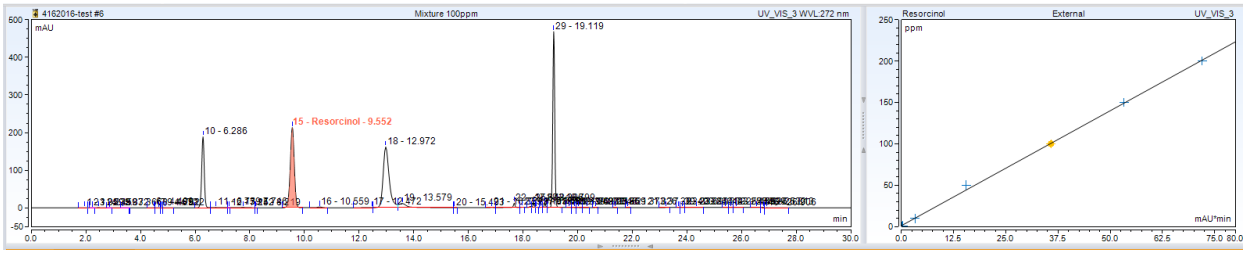


**Figure 63.** Mixture (phenol, hydroquinone, catechol and resorcinol at 100 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.

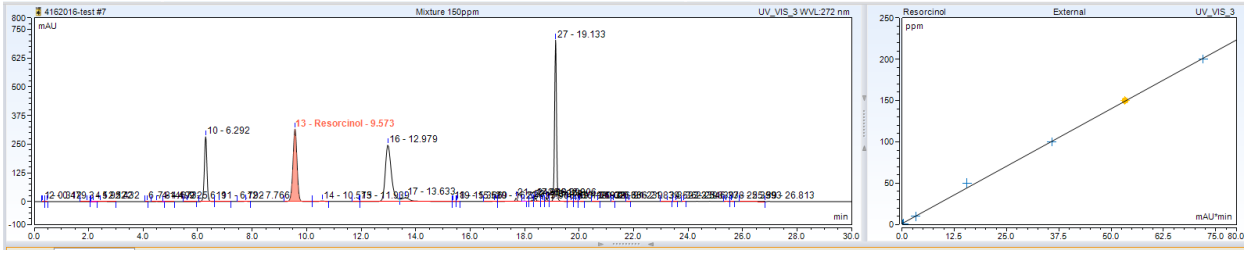


**Figure 64.** Mixture (phenol, hydroquinone, catechol and resorcinol at 150 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.

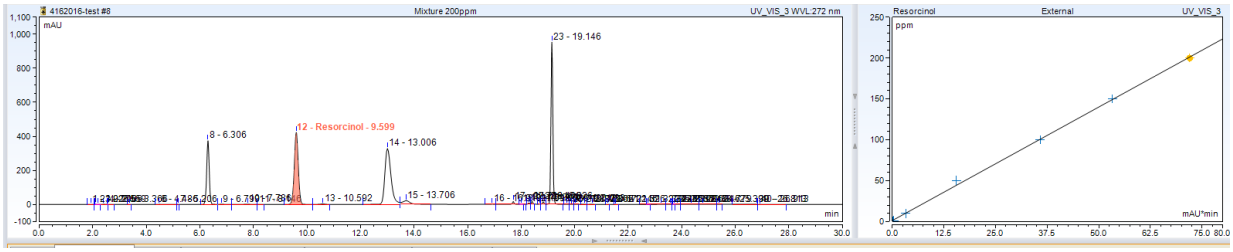


**Figure 65.** Mixture (phenol, hydroquinone, catechol and resorcinol at 200 mg/L each) absorbance under 272nm wavelength, which was used for resorcinol calibration.