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Evaluation of an anaerobic granule biosensor for upset early warning detection

by

Xi Jiang

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering (Environmental Engineering)

Program of Study Committee:
Timothy G. Ellis, Major Professor
Shihwu Sung
Thomas E. Loynachan

Iowa State University

Ames, Iowa

2008

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

In this study a porous pot reactor was used to simulate an activated sludge stage and an anaerobic granule biosensor (AGB), as an upset early warning system for activated sludge treatment process, was configured in a down-flow mode with a side-stream of the influent wastewater fed to the top. The hydraulic retention time (HRT) for the porous pot and AGB systems was 12 hours and 6 hours, respectively. The influent was made up of synthetic wastewater. In the first group of toxic loading tests, the prepared cupric chloride solutions were separately injected into both the porous pot and AGB systems at six different concentrations (5, 10, 15, 20, 30, and 45mg Cu^{2+}/L), and phenol solutions were used at four different concentrations (10, 20, 30, and 45mg phenol/L) in the second group of toxic loading tests. In addition, a control study was included for reference. The porous pot reactor and AGB were monitored for pH, ORP, COD, solids, and ammonia concentrations following a simulated toxic event. The COD removal efficiency of porous pot system decreased dramatically in response to the addition of Cu^{2+} and phenol. The greatest decrease in COD removal efficiency was from 92% to 52% at a Cu^{2+} concentration of 30 mg/L, and was from 98% to 76% at a phenol concentration of 45mg/L. The longest time of COD removal efficiency continuous decreasing was 23 hours at a Cu^{2+} concentration of 45 mg/L, and 7 hours at a phenol concentration of 10mg/L. Similar to the response in COD removal, the ammonia removal efficiency decreased significantly after the Cu^{2+} shock loading. As the porous pot reactor was challenged with the Cu^{2+} or phenol shock load, the AGB received an equivalent dose. The most sensitive parameter reflecting the toxic shock load in the AGB was the ORP. The ORP changed more rapidly and uniformly than the pH over both the range of Cu^{2+} concentrations and phenol concentration tested. The shortest response time to

peak was 1 hour after Cu^{2+} injection at 5 and10 mg/L, and was 1 hour after phenol injection at 10, 20, and 45mg/L. The longest response time was 5 hours at 15 and 30 mg/L during Cu^{2+} shock loading tests, and was 3 hours at 30mg/L during phenol shock loading test. Surprisingly, the change of pH in the AGB following Cu^{2+} or phenol addition was not as noticeable and reliable as the change in the ORP.

CHAPTER 1

GENERAL INTRODUCTION

Introduction

Activated sludge treatment, which has certain advantages of high treatment efficiency, operating simplicity and low-cost maintenance, is successfully and widely used in many wastewater treatment plants nowadays. Nevertheless, this biological treatment unit is vulnerable to discharges of wastewater containing toxic organic or inorganic substances. Toxic or inhibitory compounds at concentrations above a certain threshold may cause poor performance and reduce the quality of the treated wastewater. Thus, a new approach which could potentially be used to detect toxic conditions in the wastewater before upset wastewater treatment plant is becoming an essential need. The objective of this study is to evaluate the use of anaerobic granules, such as those cultured in a static granular bed reactor (SGBR) or up-flow anaerobic sludge blanket (UASB), in an on-line device to detect toxic conditions in the influent to an activated sludge or other biological treatment system. The concept of using anaerobic granules in a biosensor utilizes the ability of the community of microorganisms in an anaerobic environment and the method in which they break down complex organic compounds to volatile acids and, ultimately, acetate and hydrogen. Since the methanogens, which convert acetate and hydrogen to methane and CO_2, are often the most sensitive microbial population in the "bucket brigade," any toxic event will cause an accumulation of acids in the system. The result of the acid accumulation is often a drop in pH, increase in oxidation-reduction potential (ORP), and drop in methane production. Any of these process parameters can be monitored to warn of a toxic event. In this study a porous

pot reactor was used to simulate an activated sludge bioreactor and an anaerobic granule biosensor (AGB) was used as an upset early warning system. The AGB was configured in a down-flow mode with a side-stream of the porous pot influent wastewater fed to the top. The hydraulic retention time (HRT) for the porous pot and AGB systems was 12 hours and 6 hours, respectively. The influent was made up of synthetic wastewater. In the first group of toxic loading tests, the prepared cupric chloride solutions were separately injected into both the porous pot and AGB systems at six different concentrations (5, 10, 15, 20, 30, and 45mg Cu^{2+}/L), and phenol solutions were used at four different concentrations (10, 20, 30, and 45mg phenol/L) in the second group of toxic loading tests. In addition, a control reactor was included for reference. The porous pot reactor and AGB were monitored for pH, ORP, COD, solids, and ammonia concentrations following a simulated toxic event. Both of the COD and ammonia removal efficiency in the porous pot reactor decreased dramatically in response to the addition of Cu^{2+} and phenol during the two groups of shock loading tests. The most sensitive parameter reflecting the toxic loading in the AGB was the ORP. The ORP changed more rapidly and uniformly than the pH over the range of Cu^{2+} and phenol concentrations tested. The shortest response time to the peak was 1 hour after Cu^{2+} injection and phenol injection during the two groups of tests.

Thesis Organization

This thesis included a paper to be submitted to WEFTEC 2008 Annual Conference, Chicago. To make a better understand of the paper, in the thesis, chapters of general introduction/conclusion and literature review were also presented to give readers boarder backgrounds about this research study.

CHAPTER 2

LITERATURE REVIEW

Aerobic treatment process of wastewater

Aerobic treatment processes have been commonly employed for the secondary-treatment of municipal and industry wastewater treatment plants for decades, and have proven to be a stable and reliable technology. Aerobic treatment is a natural process, which requires a mechanism for injecting enough air to provide an oxygen-sufficient environment for bacteria and other microbes in the system to break down and digest the wastewater.

Though there are many designs for aerobic treatment processes, most of them have the following features in common, pretreatment of reducing the clogging solids, aeration process, settling for suspended growth, and final treatment/disinfection (Seabloom et al., 2005). The two typical aerobic treatment designs are suspended growth units and attached growth units. Suspended growth systems, e.g. activated sludge, are the most common kind of aerobic treatment systems, include a bioreactor called an aeration chamber/basin where the introduced air is mixed with the wastewater, and the oxygen-sufficient condition supports the growth of aerobic bacteria that utilize the organic matter in the wastewater as electron donors. Since the bacteria grow as they are freely suspended in the liquid and air mixture without attaching to any media surface, it is named a suspended growth process. Attached growth units, such as trickling filters and rotating biological contactors, have a media surface which is alternately exposed to the wastewater and air providing an area for bacteria and other microorganisms to attach themselves. Though this aerobic treatment unit is less widely used than suspend growth systems, it does have some advantages. For example, there is no need to

use the power to force the air into the unit and the microbial solids are easier to maintain within the system at long solids retention times, allowing for higher removal efficiencies.

Aerobic treatment process as can rapidly degrade organic compounds, reduce suspend solids and, have a high level of wastewater treatment efficiency. There are some problems and limitations as well, such as the need of power to inject air; the routine management of biomass (sludge) wastage; the requirement of trained maintenance person. In addition, aerobic treatment processes in municipal and industrial facilities are vulnerable to sudden changes in influent composition caused by organic or inorganic toxic shock loadings. For many municipal and industrial wastewater treatment plants, transient upsets resulting from influent disturbance to activated sludge and other aerobic biological treatment systems are a crucial issue (Love and Bott, 2000). Furthermore, the operators are often restrained to function in a reactive rather than a proactive mode to this problem, for which a source, cause, and effect are usually not correlated (Berthouex and Fan, 1986). Thus, upset early warning devices which could potentially be used to detect toxic conditions in the influent before overall performance of aerobic treatment process are becoming an essential need.

Activated sludge performance and benefits

Reynolds (1982) wrote the term “activated” to describe the reactive feature of the biological solids (Seabloom et al., 2005). Activated sludge is a heterogenous community of microorganisms including a mixture of different bacterial strains, rotifers, protozoa and fungi, in addition, some metazoa, such as nematode worms, may be present as well. Each of the species has its role in the wastewater treatment process, like the bacteria which are

responsible for assimilating most of the organic compounds, whereas the protozoa and rotifers play a very important role of removing the dispersed bacteria. The species of microorganism which dominates in the activated sludge system depends on environmental conditions, treatment process design and the characteristic of the secondary influent wastewater (Water Environment Association, 1987).

The activated sludge process is a biological wastewater treatment technique where a mixture of wastewater and biological sludge is sufficient aerated. The process converts most organic wastes to more stable inorganic forms, and during this process much of the soluble and colloidal organic compounds are metabolized by the community of microorganisms to carbon dioxide and water. As the microorganisms grow and are aerated thoroughly with the organic materials as food, the individual organisms flocculate to form an inactive mass of biologic floc called activate sludge, and this is the reason why we name this process as a activated sludge system (Davis and Cornwell, 1998).

Due to the different characteristics of influent wastewater and environmental conditions and limitations, there are many type of designs for activated sludge processes, such as conventional plug-flow, complete-mix, step-feed aeration, contact stabilization, high-rate aeration, sequencing batch reactor, single-stage nitrification and separate stage nitrification. The typical schematic diagram for conventional activated sludge treatment process is shown in figure 1.

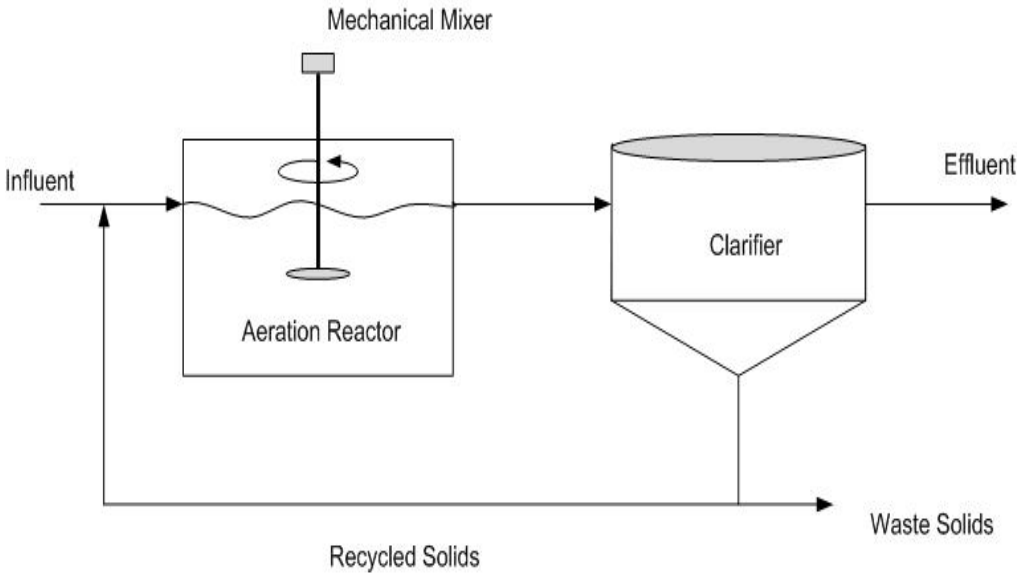

Figure I. A schematic diagram of conventional activated sludge treatment process

The aeration basin is a long and narrow tank that has enough volume to provide a 6 to 8 hours contact period before the wastewater flows to the secondary clarifier. In the secondary clarifier, the biomass is separated from the liquid stream and appropriate portion of this biological sludge is wasted and the remainder returned to the head of the aeration basin, just as figure 1 shows.

Activated sludge process is generally employed to reduce the organic and inorganic compounds in wastewater treatment plants nowadays. It has certain advantages of high level treatment efficiency, low-cost maintenance and operating simplicity (Pernetti et al., 2003), but this process has some limitations and disadvantages as well. For example, the traditional continuous flow design of activated sludge process has serious difficulties to meet the stipulated standards, and this biological treatment process is

vulnerable to the discharge of wastewater with complex organic/inorganic chemicals, any harmful chemicals with certain amount may cause the failure of the performance (Mohan et al., 2003).

Anaerobic treatment process of wastewater

Totzke (1994) reported that during the period from 1989 to 1994, the number of non-lagoon anaerobic devices has increased from about 300 to over 800 all over the world.

And in a recent record shows that there were 1215 full-scale high rate anaerobic reactors have been built for the treatment of industrial wastewater since the 1970's around the world (Frankin, 2001). All the records showed above presents that the application of anaerobic treatment processes would be more and more widely worldwide. One of the most successful anaerobic system used in the existing full-scale wastewater treatment plant is upflow anaerobic sludge blanket (UASB) reactors (Lettinga et al., 1980), which is even for the treatment of phenols and phenolic wastewaters in industry (Gali S. Veeresh, et al., 2005). There are also some other common anaerobic treatment processes, like anaerobic sequencing batch reactors (SBRs) (Wirtz and Dague, 1996) and expanded granular sludge bed (EGSB) reactors (Lettinga et al., 1994). Many of these anaerobic systems use anaerobic granular sludge to perform treatment process (Cassidy and Belia, 2005). Anaerobic granules have a higher density than aerobic flocs, which will allow reactors to have better biomass maintenance and settling features (Van Loosdrecht et al., 1995). Though the COD treatment efficiency is mainly dependen on the types of wastewater, the removal efficency of biodegradable COD by UASB in a full scale is usually above 85% or even up to 90% (Franklin, 2001). This high COD removal efficiency explains why there are over 900 UASB

reactors have been built all over the world (Alves et al., 2000). The applications of anaerobic technology are expending very fast, which not only treat effluents from industry and chemical factories, but also landfill leachates, textile industry wastewater and even for treatment of domestic wastewater in warm areas.

Compared with aerobic treatment process, anaerobic treatment methods have many advantages, what we most familiar with are much less production of biomass and reduction of biomass disposal cost; less installation space requirement; energy conservation and minimization of maintenance (R.E.Speece, 1996). Anaerobic technologies also have some other merits compared with aerobic process. For example, some organic contaminants are toxic and volatile, when they were stripped from the wastewater during the aerobic treatment process before they biodegraded, they will pollute the air, but this drawback can be avoid by utilizing the anaerobic technology (R.E.Speece, 1996).

Not limited to these advantages listed above, the most significant meaning of anaerobic process for this research study is that anaerobic systems produce acids which accumulate under environmental stress (toxic event) resulting in a rapid and noticeable drop in pH, increase in ORP, and drop in methane production shortly after exposure, and any of these process parameters can be monitored to warn of a toxic event. It is the reason why in this study we selected anaerobic granules from high rate anaerobic treatment systems as an on-line upset early warning device to prevent toxic conditions for activated sludge or other biological treatment systems.

Static Granule Bed Reactor (SGBR) performance and benefits

Static Granule Bed Reactor (SGBR) is a new anaerobic biological treatment process and was developed by Ellis and Mach in the Civil, Construction and Environmental Engineering Department at Iowa State University (Mach and Ellis, 2000). Static Granule Bed Reactor is a high rate, down flow mode anaerobic treatment system. The lab scale SGBE (Figure 2) showed as below.

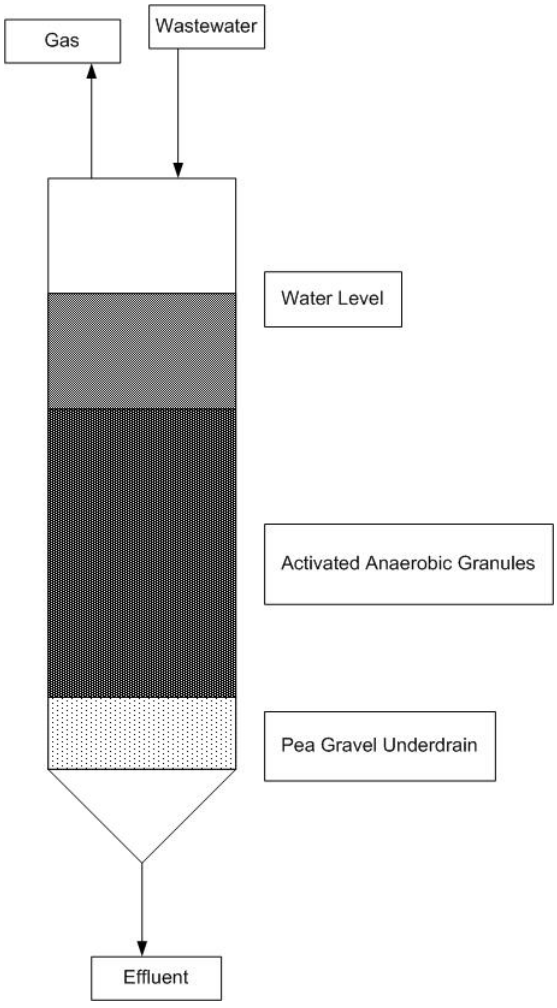

Figure II. Static Granular Bed Reactor (SGBR) schematic diagram

As shows above, the reactor headspace is empty without any separation device. The pea gravel underdrain in the bottom is used to separate solids, liquids and gas before discharging the effluent, with no requirement of backwashing systems. The SGBR system has ability to maintain high solids retention times (SRTs) and no need to changes in hydraulic retention times (HRTs) (Jung et al., 2002).

The previous study results indicate that the application of SGBR is practical and successful. The COD removal of SGBR system is over 90% with effluent Total Suspended Solids (TSS) concentration less than 100mg/l when treating different kinds of wastewaters (Mach and Ellis, 2000; and Mach et al., 2003). Effluent five-day carbonaceous biochemical oxygen demand ($CBOD_5$) could be reduced to less than 30mg/l (Mach, 2000). Mach and Ellis (2000) showed the SGBR could successfully treat a synthetic, non-fat, dry milk wastewater with COD removals above 95% by using two different SGBR heights to width ratios. In another study, Evans and Ellis (2003) compared the synthetic wastewater treatment performance of SGBR with UASB, and the SGBR had a significant higher performance of COD removal (averaging 90.7%) than UASB average COD removal (77.5%) at eight hours HRT. Also, in this study, the SGBR was proven to be a feasible alternative high-rate anaerobic wastewater treatment method at high organic loading rates of about 10gCOD/ (L.d) (Evans and Ellis, 2003).

In this study, the concept of using anaerobic granules found in SGBR utilizes the ability of the community of microorganisms in an anaerobic environment and the method in which they break down complex organic compounds to volatile acids and, ultimately, acetate and hydrogen. Since the methanogens, which convert acetate and hydrogen to methane and CO_2,

are often the most sensitive microbial population in the "bucket brigade," any toxic event will cause an accumulation of acids in the system. The result of the acid accumulation is often a drop in pH, increase in ORP, and drop in methane production. Any of these process parameters can be monitored to warn of a toxic event.

Recent developments in biosensor techniques

Organic and inorganic toxic compounds, such as copper, lead, mercury, formaldehyde, and phenol, present in many industry wastewaters like leather tanning, automobile, petroleum, and textiles dying are known to cause serious pollution problems to the receiving aquatic environment (Pamukoglu and Kargi, 2006). Monitoring of contaminants in the water is a significant technical and instrumental component in understanding and avoiding the potential effect on water ecology and human health (K.R.Rogers, 2006). Especially to biological treatment systems in domestic and industrial wastewater treatment plants, which are vulnerable to changes in influent caused by the shock loading of toxic compounds or biogenic nutrients, upset early warning devices, like biosensors or bioassays, are essential and crucial for avoiding upset events (Love and Bott, 2000). At the same time, the new technologies of monitoring toxicants in aquatic environment have become more and more concerned in recent years.

Over the past decades, biosensors have been applied in a variety of areas. (Tsai and Doong, 2004). Two common used methods are biosensors and bioanalytical devices, which appear well suited to complement standard analytical methods applying for many environmental monitoring situations (K.R.Rogers, 2006). Biosensors are integrated devices, which combine

a biological recognition component with a physicochemical detection system (Transducer system) to respond to an analyte regarding a physiological or biochemical change in a system. The biological recognition element can be a tissue, microorganisms, enzymes, antibodies, whole cell, a community of cells, etc. The transducer system works in a physicochemical way (optical, piezometric, electrochemical, etc.) to transform the biological recognition signal into a useable output signal that can be more easily measured and quantified.

Biosensors can be classified into different types according to either the biological element or the detection element. According to the biological element, they can be categorized as enzyme-based biosensors, antibody-based biosensors, cell-based biosensors, DNA biosensors, receptor-based biosensors, etc. (K.R.Rogers, 2006). Meanwhile, they can be categorized as electrochemical, optical, piezometric and thermal sensors based on the detection system.

In recent years, many methods based on biosensors and bioassays device have been developed for detecting and quantifying of toxic ion on activated sludge systems (Pamukoglu and Kargi, 2006). Some of these methods are according to inhibition of enzymatic activity (Strotmann et al., 1992); respiratory activities of the bacteria (Hu et al., 2004); kinetics of bacterial activities (Cabrero et al., 1998). Some research has been performed concerning changes in the composition of the microbial community in the presence of heavy metals, like copper (Nicolau et al., 2005). Love and Bott project team (2000), identified criteria that should be addressed by an ideal upset early warning device. The criteria include rapid response, easy to operate, distinguishable response for influent conditions, and function over a broad spectrum, etc., totally 14 criteria. No one device satisfies all of these criteria,

nevertheless we can get some general information of each kind of upset early warning device's advantage and disadvantage from the following table made by Love and Bott, 2000.

Criterion	Microcalorimetry	pH titration/alkalinity assays	Respirometry	Microtox™	Whole cell sensors: native	Whole cell GEM sensors: catabolism-based	Whole cell GEM sensors: stress-based	Whole cell GEM sensors: growth state-based	Molecular sensors: enzyme-based	Molecular sensors: immunolgical-based	Molecular sensors: nucleic acid-based
1-overall toxicity	+	+	+	+	+	-	+	+	+/-	+/-	+
2-predict process upset	?	+	?	-/?	-/?	-/?	-/?	-/?	?	?	?
2-identify source	-	-	-	?	-	+	?	-	+/?	+/?	+/?
4-rapid	+	-	-	-	+	+	+	+	+	+	?
5-anaerobic/aerobic	?	+	+/?	-	+/-	-	-	-	+	+	+
6-dose:response	?	?	+	+	+/?	+	+	+	+	+	+
7-depict corrective action	?	?	?	?	?	?	?	?	?	?	?
8-effort to operate/maintain	?	+/?	?	+	?	?	?	?	?	?	?
9-accurate interpretation	+	-	+	+	+	+	+	+	+	?	?
10-function in complex matrix	?	+	+	-	?	?	?	?	?	?	?
11-cost effective	?	?	?	?	?	?	?	?	?	?	?
12-detect false positives/negatives	-/?	-	+/-	?	+	-	-	-	+/-	+/-	+/-
13-active/sleep mode switching	+	+	+	+	?	?	?	?	?	?	?
14-commercializable	+	+	+	+	?	?	?	?	?	?	?

Table I. Summary Evaluation of Reviewed Devices Relative to the Ideal Criteria

(Love and Bott, 2000)

"+" indicates that device meets the criteria, "-" indicates that the device dose not meet the criteria, and "?" indicates that further research is needed to fully evaluate whether or not the device meets the criteria. Some variations within devices yield multiple responses for a single criterion.

The new type of biosensor developed in this study use anaerobic granules, such as found in UASB or SGBR systems, as an on-line device to detect toxic conditions in the influent to an activated sludge or other biological treatment systems. The anaerobic granule in the biosensor utilizes the ability of the community of microorganisms in an anaerobic environment and the method in which they break down complex organic compounds to volatile acids and, ultimately, acetate and hydrogen. Since the methanogens, which convert acetate and hydrogen to methane and CO_2, are often the most sensitive microbial population in the "bucket brigade," any toxic event will cause an accumulation of acids in the system. The result of the acid accumulation is often a drop in pH, increase in oxidation-reduction potential (ORP), and drop in methane production. Any of these process parameters can be monitored to warn of a toxic event. In this study, the anaerobic granule biosensor (AGB) seeks to address the difficulties with existing biosensor configurations, like complexity of construction, low sensitivity to environmental stress, robustness, and high cost. The advantages of this new approach is that consortia of microorganisms within the granule has the necessary diversity to be sensitive to a wide rang of environmental stresses, and also potentially has more robust and application to actual working conditions. Moreover, the biosensor would be economical (anaerobic granules are easily obtained and low cost), highly sensitive to environmental stress, easily replicated (anaerobic granules are relatively uniform in microbial composition), and the response of the biosensor is expected to be rapid.

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CHAPTER 3

EVALUATION OF AN ANAEROBIC GRANULE BIOSENSOR FOR UPSET EARLY WARNING DETECTION

A paper to be submitted to WEFTEC 2008 Annual Conference

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ABSTRACT

The objective of this study was to evaluate the use of anaerobic granules in an on-line device to detect toxic conditions in the influent to a biological wastewater treatment system. In this study a porous pot reactor was used to simulate the activated sludge stage of a wastewater treatment plant, and an anaerobic granule biosensor (AGB) was evaluated as an upset early warning device (UEWD). In the first group of toxic loading tests, the prepared cupric chloride solutions were separately injected into both the porous pot and AGB systems at six different concentrations, and phenol solutions were used at four different concentrations in the second group of tests. The results showed the porous pot chemical oxygen demand (COD) and ammonia-N removal efficiency decreased dramatically in response to the addition of Cu^{2+} and phenol. The most sensitive parameter was the oxidation-reduction potential (ORP) in the AGB, which suggests that the AGB could potentially be used as an on-line UEWD.

KEYWORDS: Biosensor, anaerobic granules, activated sludge, copper ion, phenol, COD removal, ORP, response time

INTRODUCTION

Industrial and domestic wastewater must be sufficiently treated at a municipal wastewater treatment plant (WWTP) before discharge to a receiving stream. Activated sludge treatment is a common method for organic and inorganic pollutant removal in biological (secondary) wastewater treatment systems (Mimma et al., 2003). Any biological treatment system, however, is vulnerable to discharges of industrial wastewater with various organic and inorganic toxic compounds (Jonsson et al., 2000). Toxic compounds above a certain threshold concentration may cause poor performance of activated sludge systems or other biological treatment processes and reduce the quality of the treated wastewater at the same time (Dalzell et al., 2002). It is very important to monitor the efficiency of the various treatment systems used by industrial and municipal plants to prevent toxic substances from causing system failure (Ciccotelli et al., 1998), (Wong et al., 1995). Thus, there is an urgent need for wastewater treatment plant operations personnel to be able to detect toxic conditions in the influent before upset conditions occur in the biological unit processes.

During the past several decades, many methods based on biosensors or bioassays have been used for detecting and quantifying toxicity or inhibition in activated sludge systems or other biological systems (Pamukoglu and Kargi, 2006). Farre (2003) has reviewed different bioassays and recently developed biosensors on acute toxicity measurements. Some of these methods were according to enzyme inhibition (Dalzell et al., 2002) and respiratory activities of the bacteria (Hu et al., 2004). Some research has looked at changes in the composition of the microbial community in the presence of heavy metals, like copper (II) (Nicolau et al., 2005), or engineered bacteria based biosensors in the presence of heavy metals (Leth et al.,

2002). Monitoring the toxicity of influent conditions in WWTP using biosensor or bioassays could possibly provide upset early warning to protect the performance of biological wastewater treatment systems.

A recent survey of on-line upset early warning devices revealed that while there are many commercially available systems, very few installations have had success in operating and maintaining these devices long term (Standley and Ellis, 2003). It is significant to develop a suite of standard direct toxicity assessment bioassays which are precise, traceable and can be used to certify measurements (Dalzell et al., 2002). In 2000, Love and Bott project team identified criteria that should be addressed by an ideal upset early warning device. The criteria include rapid response, simple and convenient operation, distinguishable response for influent conditions, function over a broad spectrum, and be inherently capable of commercialization, etc., totally 14 criteria. Though no one biosensor or bioassay is likely to meet all of these criteria, it might be possible to satisfy more criteria by integrating more than one device as part of an UEWD (Love and Bott, 2000).

In this study, a new type of biosensor was developed by using anaerobic granules, such as found in an up-flow anaerobic sludge blanket (UASB) or static granular bed reactor (SGBR), in an on-line device to detect toxic conditions in the influent to an activated sludge or other biological treatment system. The concept of using anaerobic granules in a biosensor takes advantage of the ability of the community of microorganisms in an anaerobic environment and the method in which they break down complex organic compounds to volatile acids and, ultimately, acetate and hydrogen. Since the methanogens, which convert acetate and

hydrogen to methane and CO_2, are often the most sensitive microbial population in the "bucket brigade," any toxic event will cause an accumulation of intermediates, namely acids, in the system. The result of the acid accumulation is often a drop in pH, increase in ORP, and drop in methane production. Any of these process parameters can be monitored in an anaerobic UEWD to warn of a toxic event. The AGB seeks to address the difficulties with existing biosensor configurations, like complexity of construction, low sensitivity to environmental stress, robustness, and high cost. The advantages of this new approach is that consortia of microorganisms within the granule has the necessary diversity to be sensitive to a wide rang of environmental stresses, and also potentially has more robust and applicable to actual working conditions. Moreover, the biosensor would be economical (anaerobic granules are relatively inexpensive and easy to obtain), highly sensitive to environmental stress, easily replicated (assuming that anaerobic granules are relatively uniform in microbial composition), and the response of the biosensor is expected to be rapid.

MATERIALS AND METHODS

Laboratory-scale Activated Sludge Unit and AGB System

In this study, a porous pot reactor (Figure 1) was used to simulate a laboratory-scale activated sludge unit with a solids retention time (SRT) of 12 days. It consisted of an external Plexiglas cylinder that surrounded a second cylinder made of stainless steel mesh screen with a pore size of 65 μ. The outer cylinder had an overflow to maintain the sludge volume of the reactor at 1.3 L. Aeration was provided to the biomass in the reactor via a diffuser stone connected to the compressed air line in the laboratory. Seed biomass was collected from the solids contact basin at the Ames Water Pollution Control Facility (Ames, Iowa), and the mixed liquor

suspended solid (MLSS) and pH of the biomass were maintained at a concentration of 2615 ± 261mg/L and 7.4 ± 0.3, respectively. The hydraulic retention time (HRT) of the porous pot reactor was fixed at 12 h. Meanwhile, an AGB system employed in this study was configured in a down-flow mode with a side-stream of the porous pot influent wastewater fed to the top (Figure 1). The volume of anaerobic granules and supernatant in the AGB system was approximately 0.7 and 0.3 L, respectively. The hydraulic retention time (HRT) for the AGB system was fixed at 6 h. Seed anaerobic granules were collected from a Static Granular Bed Reactor (SGBR), which was operated in the Iowa State University Environmental Laboratory (Ames, Iowa).

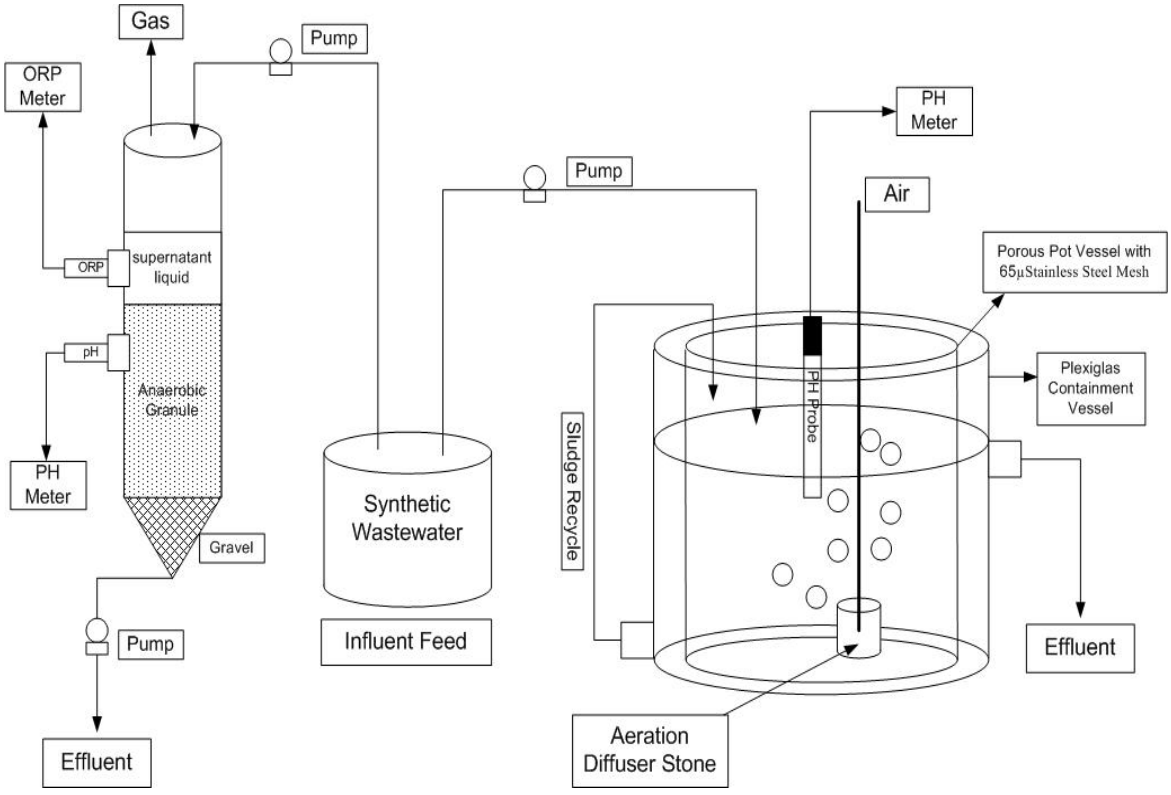

Figure 1. Schematic of porous pot reactor and AGB system.

Synthetic Wastewater Composition

The influent was synthetic wastewater stored at 4^{o}C until it was fed to the porous pot and AGB reactors. The composition is listed in Table 1.

Table 1. Synthetic Wastewater Components

Macronutrient	**Concentration (mg/l)**
NH_4Cl	24.5
Nutrient Broth	42.444
Lauric Acid	14.008
Potato Starch	20.272
Non-fat Dried Milk	326.384
Sodium Acetate	21.248
Micronutrient	**Concentration (mg/l)**
$NiSO_46H_2O$	0.4
$ZnCl_2$	0.248
$CuCl_22H_2O$	0.448
$CoCl_26H_2O$	0.072
$MnSO_4H_2O$	0.088
EDTA	0.296
$Na_2MnO_42H_2O$	0.024
$NaHCO_3$	40
$K_3PO_4H_2O$	12
$FeSO_47H_2O$	2

The physical parameters such as total and volatile suspend solids (TSS & VSS) and chemical parameters such as pH and Total COD of the synthetic wastewater are listed in Table 2.

Table 2. Physical and chemical parameters of synthetic wastewater.

Parameters	**Measured Value[1]**	**Units**
pH	7.5 ± 0.2	
Total COD	374 ± 33	mg/L
TSS	127 ± 21	mg/L
VSS	112 ± 19	mg/L

[1]Mean ± Standard Deviation of 10 synthetic wastewater (influent) samples taken at the beginning of each toxic loading test.

Shock Loading Chemicals

In the first group of toxic shock loading tests, copper (II) was selected due to its common presence in industrial wastewater and known inhibitory characteristics. Cupric chloride ($CuCl_2.2H_20$) solutions were made in distilled water with representative amounts of copper (II) ion, and the prepared solutions were separately injected into both porous pot and AGB systems during the respective toxic loading tests at six different concentrations (5, 10, 15, 20, 30, and 45 mg Cu^{2+}/L). In the second group of tests, phenol solutions were prepared and injected in the same way as the Cu^{2+} tests but at four different concentrations (10, 20, 30, and 45mg phenol/L). In addition, a control study was also included for reference (Appendix, Table A).

Experimental Procedure

The porous Pot and AGB reactors were fed the exactly same synthetic wastewater during the study, and the hydraulic retention time was fixed at 12 hours and 6 hours, respectively, by setting the flow rate of the feed wastewater pumps. The sludge age was adjusted by removing the required amount of sludge from the porous pot by using a syringe to keep the sludge age at approximately 12 days in this study. The temperature was approximately 25 ± 2^{o}C throughout the experiments. The study was performed in the order of increasing concentrations from 5 to 45 mg Cu^{2+}/L in the first group of tests and from 10 to 45 mg phenol/L in the second group of tests. We also performed a copper (II) ion and phenol free study in the same condition to observe the performance in both of the reactors as a reference. At the beginning of each toxic shock loading test, the porous pot mixed liquor suspended solid was kept around 2615 mg/L insure each test was at the same condition during the study. After injecting the representative amount of cupric chloride solution or phenol solution into both of the porous pot and AGB reactors, the observation periods were from 45 hours to 100 hours to insure the COD and ammonia-N removal percentage recovered to or close to the starting level. After each shock loading test cycle, we continuously ran both of the reactors for approximately one week to achieve steady state conditions before the next toxic loading test. The porous pot reactor and AGB were monitored for pH, ORP, COD, solids, and ammonia-N concentrations following each simulated toxic event throughout the study.

Analytical Methods

Samples were taken in duplicate and analyzed by *Standard Methods for the Examination of Water and Wastewater* (APHA et. al., 1995). Total COD influent and effluent samples were

unfiltered and measured with Methods 5220C. Total suspend solids and volatile suspend solids samples were pre-filtered (1.2 μm, Whatman GF/C1822-042) and quantified with Methods 2540D/2540E. An orion Portable pH meter with combination electrode (Model 210A) and Nessler Method (HACH, DR/3000) were used to measure porous pot pH and ammonia-nitrogen concentrations, respectively. An Orion Portable pH and ORP meter (Model 210A and Model 250A) with combination electrodes were used to measure pH and ORP of AGB reactor. Gas production from the AGB system was measured using the Cole Parmer loop-powered gas transmitter and monitor, which meters gas based on differential pressure sensing plates.

RESULTS AND DISCUSSION

Copper (II) Ion Shock Loading Effects on Porous Pot and AGB Systems

The COD removal efficiency decreased dramatically in response to the addition of Cu^{2+}. The greatest decrease in COD removal efficiency was from 92% to 52% at a Cu^{2+} concentration of 30 mg/L, and the removal efficiency continued to decrease for 19 hours following injection. The longest time of decreasing COD removal efficiency was 23 hours at a Cu^{2+} concentration of 45 mg/L. At concentrations of 20 mg/L or less, the COD removal efficiency decreased for approximately 7 hours, after which time the system started to recover from the toxic event (Figure 2).

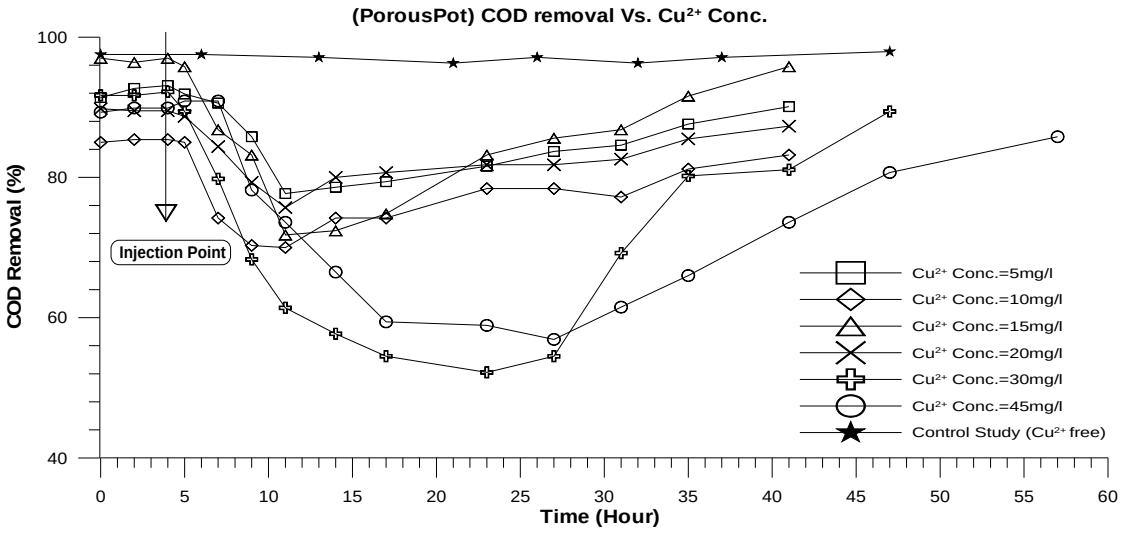

Figure 2. Porous Pot COD removal efficiency versus time at various Cu^{2+} concentrations.

Similar to the response in COD removal, the ammonia removal efficiency decreased significantly after the Cu^{2+} shock loading. Ammonia-N concentrations in the porous pot increased dramatically at the high Cu^{2+} concentration shock loading tests (30 and 45 mg/L), but during the lower concentration tests (5 and 15 mg/L), the changes were not significant (Figure 3).

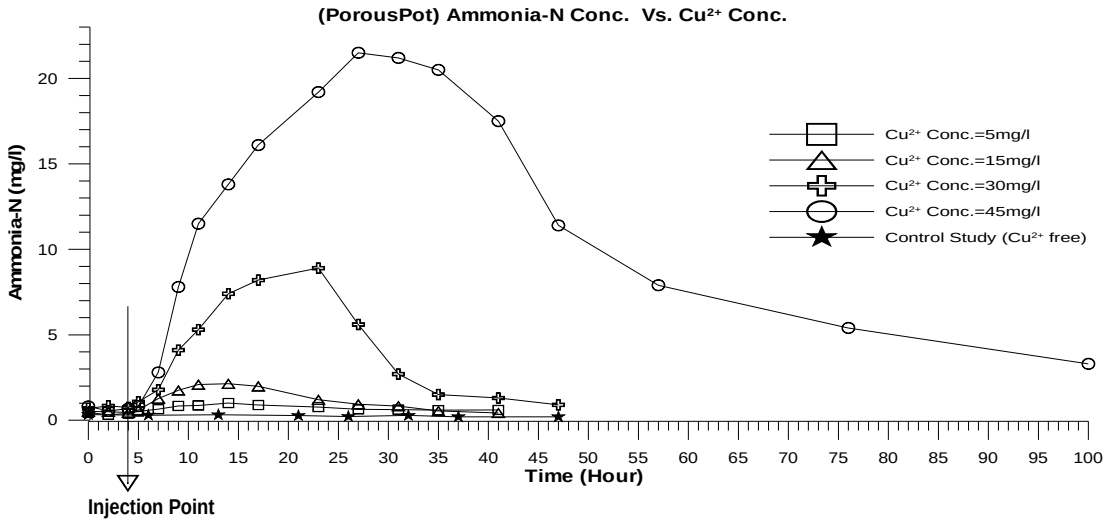

Figure 3. Porous pot ammonia-N concentration versus time at various Cu^{2+} concentrations.

During each of the Cu^{2+} injection tests, the pH of the porous pot system fluctuated within a small range. The smallest range was from 7.4 to 7.6 at a Cu^{2+} concentration of 10 mg/L, and the largest range was from 7.8 to 8.3 at a Cu^{2+} concentration of 45 mg/L. Compared to the porous pot control study pH range of 0.1, which is from 7.4 to 7.5, the porous pot pH change during the toxicity tests was not significant.

The most sensitive parameter reflecting the toxic shock load to the porous pot reactor was the ORP of the AGB. The ORP changed more rapidly and uniformly than the pH or COD removal efficiency over the range of Cu^{2+} concentrations tested (i.e., 5 to 45 mg/L). After toxic loading injection, the ORP increased immediately. The effect lasted for several hours; after which the ORP decreased back to the starting value or lower. This trend was uniform thorough all different concentrations of the toxic loading tests. The shortest response time to peak was 1 hour after injection at Cu^{2+} concentration of 5 and10 mg/L. The response time was 3 hours at Cu^{2+} concentrations of 20 and 45 mg/L, and the longest response time was 5 hours after injection at Cu^{2+} concentration of 15 and 30mg/L. The largest increase in the ORP was 20.2 mV (from -238.7 to -218.5mV) at a Cu^{2+} concentration of 20 mg/L, and the smallest increase was 6.4 mV (from -233.4 to -227mV) at a Cu^{2+} concentration of 5 mg/L (Figure 4).

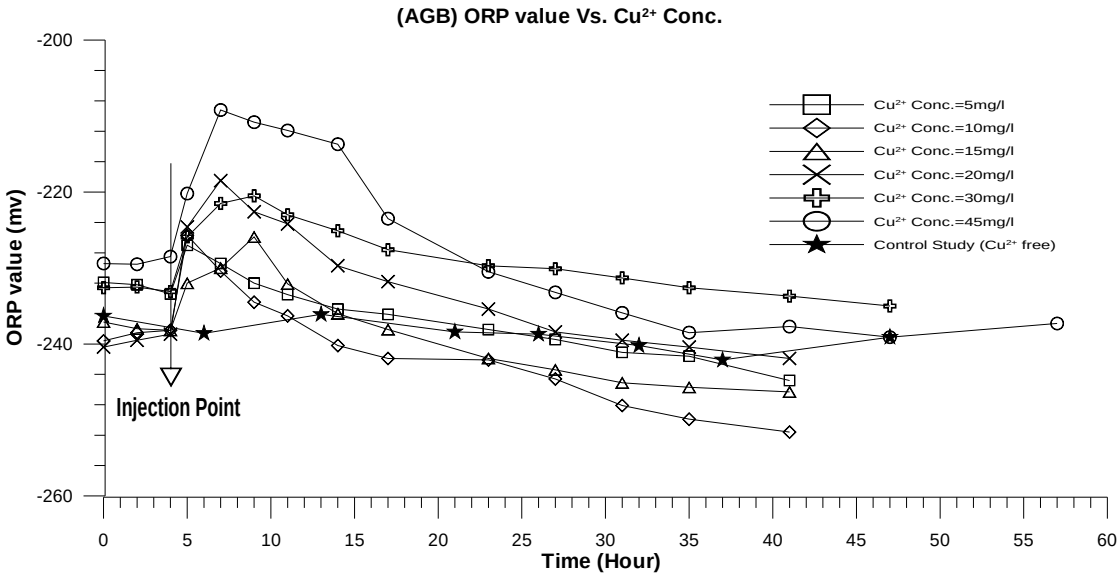

Figure 4. AGB system ORP value versus time at various Cu^{2+} concentrations.

Surprisingly, the pH response in the AGB system following Cu^{2+} addition was neither noticeable nor reliable. The largest change of AGB pH was from 6.87 to 6.65 at a Cu^{2+} concentration of 45 mg/L and the smallest change was from 6.99 to 6.86 at a Cu^{2+} concentration of 15 mg/L. This is perhaps indicative of the resilience of the anaerobic granules and their ability to shield the methanogenic bacteria which tend to reside in the interior of the granule (Baloch, et al., 2007). The dense granules could possibly have limited the diffusion of Cu^{2+} (Mu, et al., 2006), preventing exposure to the methanogens. Meanwhile, the AGB system COD removal efficiency was not affected significantly during each of the toxic loading test compared with observed ORP value. Moreover, different from observed ORP value changing trend, which was increase first, then covered back to the test start point, the changes for COD removal efficiency were fluctuate during all the test periods. The largest change was 12.7% (from 91.4% to 78.7%) at a Cu^{2+} concentration of 45 mg/L, and the smallest range was 4.3% (from 88% to 83.7%) at a Cu^{2+} concentration of 20 mg/L.

Phenol Shock Loading Effects on Porous Pot and AGB Systems

The COD removal efficiency trends with phenol as the inhibitor were similar to the first group of tests with copper II as the inhibitor. The COD removal decreased dramatically in response to the addition of phenol. The greatest decrease in COD removal efficiency was from 98% to 76% at a phenol concentration of 45 mg/L. The longest time of continuously decreasing COD removal efficiency was 5 hours at a phenol concentration of 10 mg/L. At concentrations of 20, 30 and 45mg/L, the COD removal efficiency decreased for approximately 3 hours, after then the system started to recover from the toxic events. Compared with the system recovery following the Cu^{2+} shock loading tests, the recovery trends of this group of tests occurred much earlier (Figure 5).

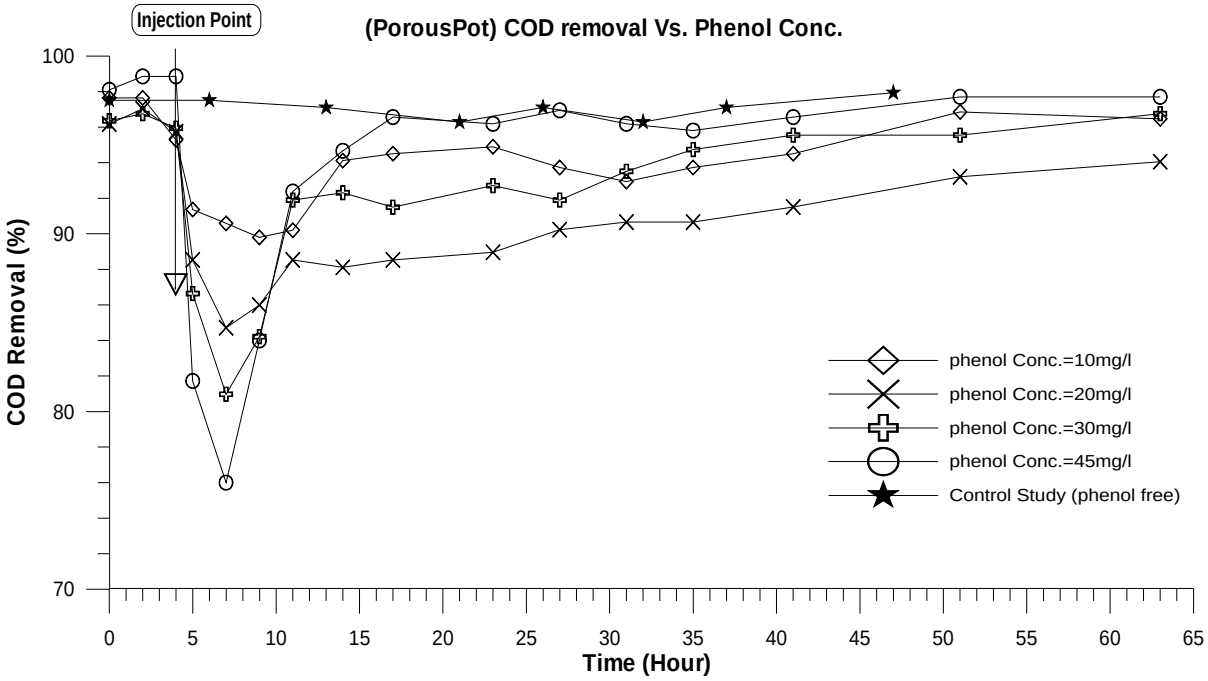

Figure 5. Porous Pot COD removal efficiency versus time at various phenol concentrations.

The ammonia-N concentration of the porous pot reactor increased rapidly and significantly after injecting the phenol solution, and then recovered back to the normal condition after several or more hours during the tests. The most dramatic increase was from 0.19 to 9.5 mg/L at phenol injection concentration of 45 mg/L. This response was different from the first group of tests with Cu^{2+}. The recovery times were much shorter at each phenol concentration of 20, 30 and 45 mg/L, but somewhat longer for the 10 mg/L injection (Figure 6).

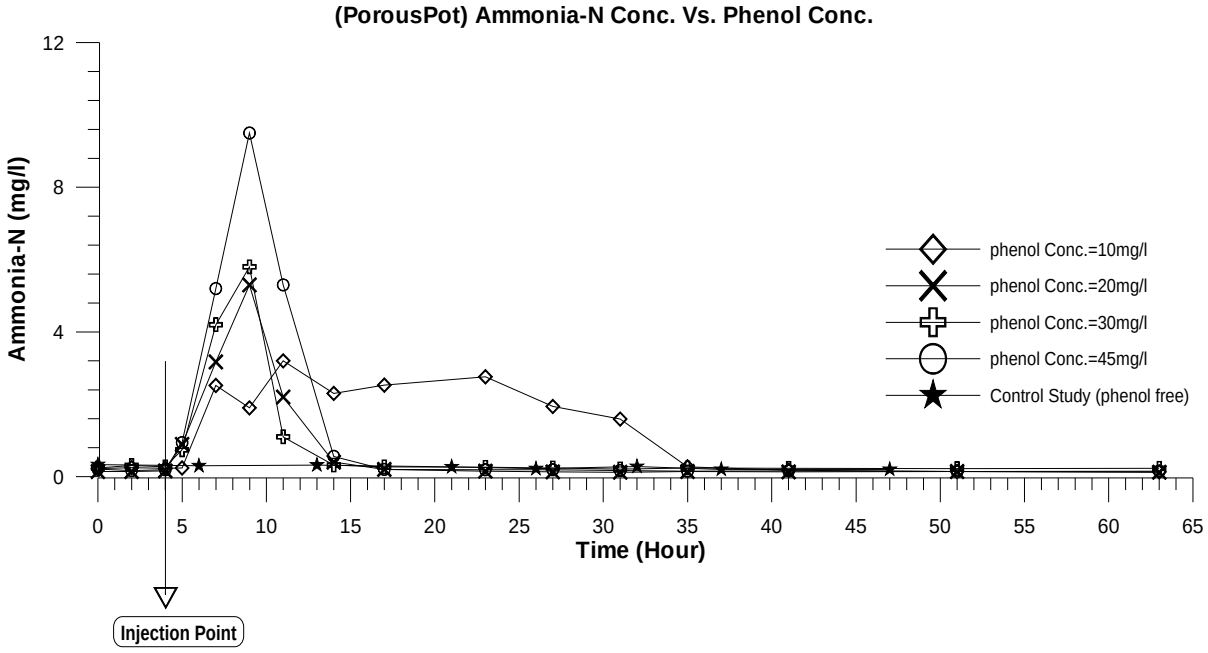

Figure 6. Porous pot ammonia-N concentration versus time at various phenol concentrations.

As was the case for the first group tests, the most sensitive parameter reflecting the toxic shock load was the ORP in the AGB. The responses of ORP in AGB system were rapid and uniform. After injecting phenol solution, the ORP increased immediately. The increase in ORP lasted from 1 to 3 h, then decreased back to the original condition or even a little lower.

This trend was uniform thorough all different phenol concentration tests. The shortest response time to peak value after injection was 1 h at a phenol concentration of 10, 20 and 45mg/L; the longest response time was 3 h at a phenol concentration of 30 mg/L. The largest increase in the ORP was 25.7 mV (from -254.1 to -228.4 mV) and the smallest increase was 15.1 mV (from -248.2 to -233.1 mV), which occurred at phenol concentrations of 20 and 45 mg/L, respectively (Figure 7).

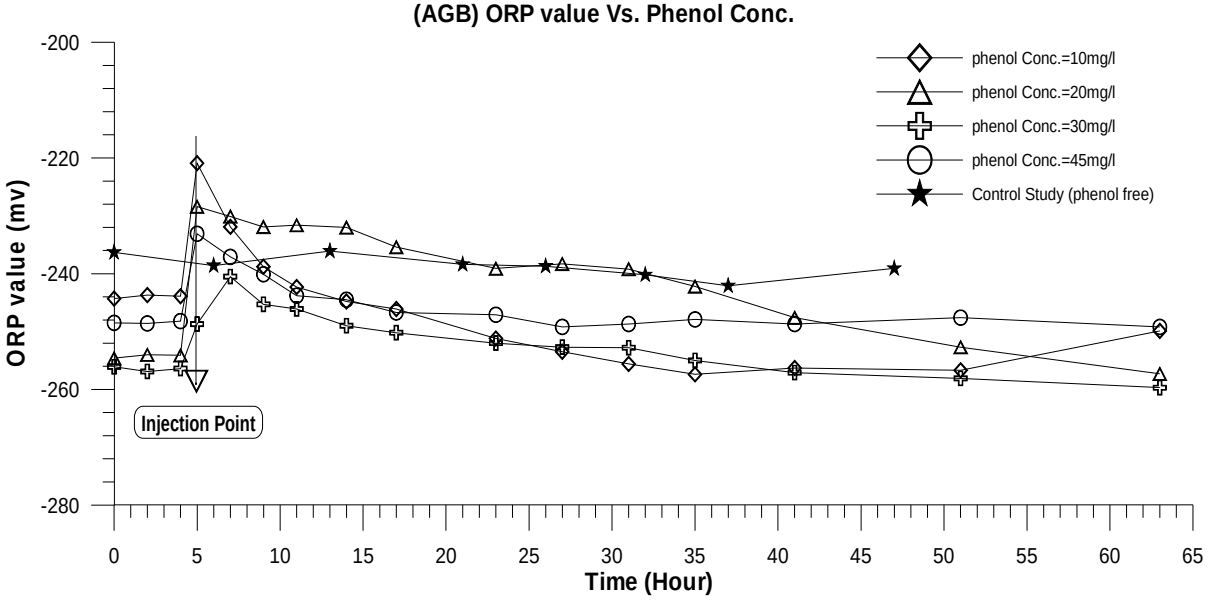

Figure 7. AGB system ORP value versus time at various phenol concentrations.

Discussion of AGB Response to Porous Pot Performance

Both of the inorganic (Cu^{2+}) and organic (phenol) shock loading test results of porous pot system showed that the most significant parameters reflecting performance were COD and ammonia-N removal efficiency, which decreased dramatically during the test especially when the toxic loading concentration was high. Meanwhile, the rapid and obvious response

in the ORP of the AGB system demonstrates the concept of using anaerobic granules in UEWD. Comparing the response of those parameters, it was shown that the most sensitive response parameter was ORP in AGB system. The response time of ORP in the AGB ranged from 1 to 5 hours in the Cu^{2+} shock loading tests and 1 to 3 hours in the phenol shock loading tests. This was a significantly more rapid response time compared to COD and ammonia-N removal efficiency response times of 7 to 23 hours and 10 to 23 hours, respectively for the Cu^{2+}. In the phenol shock loading tests, the COD and ammonia responses were observed 3 to 5 hours and 5 to 7 hours, respectively, following the phenol injection. The ORP response time was rapid and indicative of the inhibitory condition. The response of AGB system was approximately 2 to 20 hours (0 to 4 hours in phenol toxic loading tests) in advance of porous pot reactor performance response (Figure 8 and Figure 9). This would be sufficiently long to observe the upset event and do take proactive measures to protect the biomass in the system. Moreover, the ORP response of AGB system monitored continuously on-line with little operational complexity. For the COD and ammonia-N removal efficiency, we need to get the effluent sample analyzed in the laboratory, those which takes at least several hours, thus, the AGB system as a UEWD is very effective and important to secondary biological treatment process performance in WWTP.

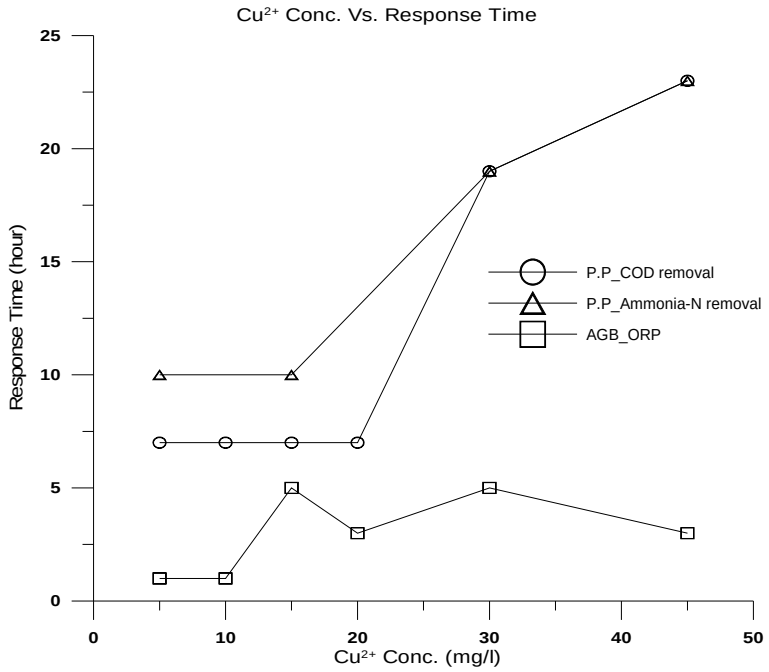

Figure 8. Cu^{2+} concentration versus response time of Porous Pot and AGB parameters.

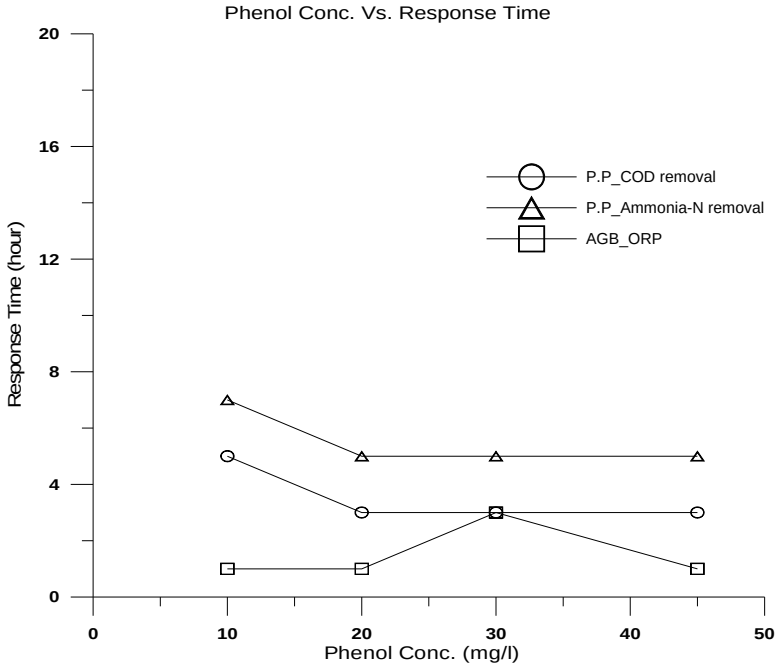

Figure 9. Phenol concentration versus response time of Porous Pot and AGB parameters.

CONCLUSIONS

In this study, the anaerobic granule biosensor was evaluated as an on-line device to detect toxic conditions in the influent to the activated sludge system. From the results we obtained, the response time of the ORP in the AGB system was 1 to 5 hours following an inorganic (Cu^{2+}) shock load and 1 to 3 hours following the organic (phenol) shock load. Both of these responses were in advance of the deterioration in the porous pot (activated sludge system) performance response, and it was a sufficient advance notice to allow proactive measures to be taken to protect the system. Meanwhile, the ORP response was much easier to obtain than the COD or ammonia-N removal efficiency response of the activated sludge system. This research supports the practicality of using anaerobic granules in an online biosensor system for upset early warning detection of toxic and inhibitory conditions in biological wastewater treatment plants.

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CHAPTER 4

GENERAL CONCLUSION

General Discussion

Results from this study showed AGB system provided a rapid response, was sensitive and could be easily obtained. The AGB was effective as an UEWD for on-line monitoring of a secondary biological treatment process performance in a WWTP. The practical and engineering significance of this study was to prevent potential upset events in biological wastewater treatment systems in WWTP.

Engineering Significance

Monitoring of contaminants in the air, water and soil is a critical issue in understanding and managing risks to human beings and the environment. Most WWTP have been designed to treat domestic wastewater without inhibitory contaminants. Nevertheless, wastewater operations personal need to be prepared for potentially toxic or inhibitory upstream discharges and unexpected pollution incidents from industry or upset events. Although an array of upset early warning deices are available, there are few well-controlled, accurate and cost-effective technologies to detect and mitigate upset events. The AGB system developed in this study is verified as an on-line and sensitive device (the methanogenic cultures in particular are thought to be some of the most sensitive organisms to environment stress). It produces a rapid response, and utilizes low-cost anaerobic granules. In addition for use in wastewater treatment plants, the potential applications of the AGB system could also be in aqueous environments, such as lakes, streams, and industrial plants.

Recommendations for Future Research

Biosensors for certain potential environmental applications continue to show advantages in areas such as protecting the nation's water supplies from harmful chemicals and bioterrorism. In this project, the anaerobic granules from a static granular bed reactor were evaluated as an on-line device to detect toxic conditions in the influent to an activated sludge or other biological treatment system. From all the test results we obtained, we can make a conclusion that the AGB system is sensitive enough and easily obtained microbial culture biosensor, which may potentially detect toxic conditions with quick response and effectively reduce the functional and economic risks of local WWTP.

The next step to make this research more complete is to test the performance of anaerobic granules on other inorganic and organic compounds, such as zinc, toluene, and pesticides. For each of the toxic or inhibitory environmental stresses imposed on the AGB, a dose-response relationship will be developed as was done in this study. Additional verification of the AGB could be in pilot and full-scale tests in municipal and industrial wastewater treatment applications.

This AGB technique for detecting inhibitory or toxic conditions has continuous shown significant potential as a UEWD. Its advantages, such as low cost, robust, and simple operation, will likely make it popular in the future development of biosensor systems for the environmental market. The biosensor system operated as simple as ORP/pH electrode in terms of selectivity, sensitivity, durability and large concentration range, may possibly be widely accepted and used for environmental toxic event monitoring.

APPENDIX A: CONTROL STUDY OF POROUS POT AND AGB MONITORED PARAMETERS

Table A. Control Study of Porous Pot and AGB monitored parameters

Time (hour)	PP_pH	PP_Ammonia-N (mg/l)	PP_COD removal %	AGB_pH	AGB_ORP (mv)	AGB_COD removal %
0	7.6	0.33	97.52	7	-236.3	81.84
6	7.5	0.3	97.52	6.97	-238.6	83.08
13	7.4	0.32	97.11	7.01	-236.1	83.91
21	7.3	0.27	96.29	7.11	-238.4	83.08
26	7.4	0.22	97.11	7.15	-238.7	81.43
32	7.3	0.28	96.29	7.21	-240.2	81.84
37	7.4	0.2	97.11	7.23	-242.1	81.84
47	7.6	0.2	97.94	7.1	-239.1	85.56

APPENDIX B: OPERATING DATA OF POROUS POT SYSTEM

Table B1. Porous Pot pH value Vs. Injecting Cu^{2+} Conc. (mg/l)

Test Time (Hour)	Cu^{2+} Conc. = 5 mg/l	10 mg/l	15 mg/l	20 mg/l	30 mg/l	45 mg/l
0	7.8	7.45	8	7.3	7.7	7.9
2	8	7.5	8.1	7.25	7.6	7.8
4	7.8	7.4	8	7.2	7.7	7.8
5	7.7	7.5	8	7.1	7.8	7.85
7	7.8	7.55	8.1	7.15	7.9	8
9	7.9	7.55	8.1	7.2	8	8.1
11	7.8	7.5	8.1	7.3	7.9	8.2
14	7.9	7.6	8	7.2	8	8.25
17	7.8	7.55	8	7.15	7.9	8.2
23	7.7	7.55	7.9	7.2	7.7	8.2
27	7.8	7.5	8	7.25	7.6	8.3
31	7.6	7.5	8.1	7.15	7.7	8.3
35	7.6	7.5	7.8	7.15	7.6	8.3
41	7.8	7.45	7.7	7.2	7.9	8.2

Table B2. Porous Pot COD removal efficiency (%) Vs. Injecting Cu^{2+} Conc. (mg/l)

Test Time (Hour)	Cu^{2+} Conc. = 5 mg/l	10 mg/l	15 mg/l	20 mg/l	30 mg/l	45 mg/l
0	91.4	85	97	89.8	91.7	89.3
2	92.7	85.4	96.4	89.5	91.7	89.9
4	93.1	85.4	97	89.5	92.2	89.9
5	91.9	85	95.8	88.7	89.4	90.9
7	90.6	74.2	86.8	84.4	79.8	90.9
9	85.8	70.3	83.2	79.3	68.3	78.2
11	77.7	70	71.8	75.7	61.4	73.6
14	78.6	74.2	72.4	80	57.7	66.5
17	79.4	74.2	74.8	80.7	54.5	59.4
23	81.6	78.4	83.2	81.8	52.2	58.9
27	83.7	78.4	85.6	81.8	54.5	56.9
31	84.6	77.2	86.8	82.6	69.2	61.5
35	87.6	81.2	91.6	85.5	80.2	66
41	90.1	83.2	95.8	87.3	81.1	73.6
47					89.4	80.7
57						85.8

Table B3. Porous Pot Effluent Ammonia_N Conc. Vs. Injecting Cu^{2+} Conc. (mg/l)

Test Time (Hour)	Cu^{2+} Conc. = 5 mg/l	15 mg/l	30 mg/l	45 mg/l
0	0.45	0.52	0.7	0.81
2	0.32	0.5	0.84	0.54
4	0.4	0.45	0.75	0.68
5	0.52	0.6	1.08	0.83
7	0.65	1.27	1.78	2.8
9	0.84	1.75	4.1	7.8
11	0.87	2.09	5.3	11.5
14	1.01	2.14	7.4	13.8
17	0.89	1.99	8.2	16.1
23	0.77	1.2	8.9	19.2
27	0.64	0.94	5.6	21.5
31	0.62	0.83	2.7	21.2
35	0.59	0.54	1.5	20.5
41	0.6	0.43	1.3	17.5
47			0.9	11.4
57				7.9
76				5.4
100				3.3

Table B4. Porous Pot pH value Vs. Injecting Phenol Conc. (mg/l)

Time (Hour)	Phenol Conc. =10 mg/l	20 mg/l	30 mg/l	45 mg/l
0	7.7	7.8	7.8	7.9
2	7.8	7.8	7.8	8
4	7.8	7.9	7.9	7.9
5	7.9	7.9	8.1	8.1
7	7.9	8	8	8.1
9	7.8	7.9	8	7.9
11	7.8	8	8	7.9
14	7.8	7.9	7.9	8
17	7.8	7.9	7.9	7.9
23	7.7	7.9	7.8	7.8
27	7.7	8	7.9	7.9
31	7.7	8	7.9	7.8
35	7.8	7.9	7.9	7.8
41	7.8	7.9	7.9	7.8
51	7.6	7.7	8	7.9
63	7.6	7.8	7.8	7.9

Table B5. Porous Pot COD removal efficiency (%) Vs. Injecting Phenol Conc. (mg/l)

Time (Hour)	Phenol Conc. =10 mg/l	20 mg/l	30 mg/l	45 mg/l
0	97.65	96.18	96.36	98.10
2	97.65	97.03	96.76	98.86
4	95.29	95.75	95.95	98.86
5	91.37	88.53	86.64	81.72
7	90.59	84.71	80.97	76.00
9	89.80	85.99	84.21	84.00
11	90.20	88.53	91.90	92.38
14	94.12	88.11	92.31	94.67
17	94.51	88.53	91.50	96.57
23	94.90	88.96	92.71	96.19
27	93.73	90.23	91.90	96.95
31	92.94	90.66	93.52	96.19
35	93.73	90.66	94.74	95.81
41	94.51	91.51	95.55	96.57
51	96.86	93.21	95.55	97.71
63	96.47	94.06	96.76	97.71

Table B6. Porous Pot Effluent Ammonia_N Conc. Vs. Injecting Phenol Conc. (mg/l)

Time (Hour)	Phenol Conc. =10 mg/l	20 mg/l	30 mg/l	45 mg/l
0	0.22	0.14	0.24	0.21
2	0.25	0.14	0.31	0.17
4	0.23	0.16	0.27	0.19
5	0.24	0.89	0.74	0.94
7	2.52	3.17	4.2	5.2
9	1.9	5.3	5.8	9.5
11	3.2	2.2	1.1	5.3
14	2.3	0.4	0.32	0.56
17	2.53	0.2	0.27	0.2
23	2.76	0.15	0.26	0.19
27	1.94	0.13	0.24	0.19
31	1.59	0.12	0.21	0.17
35	0.27	0.14	0.24	0.15
41	0.18	0.13	0.23	0.16
51	0.14	0.14	0.22	0.14
63	0.13	0.12	0.23	0.15

APPENDIX C: OPERATING DATA OF AGB SYSTEM

Table C1. AGB system pH value Vs. Injecting Cu^{2+} Conc. (mg/l)

Test Time (Hour)	Cu^{2+} Conc. = 5 mg/l	10 mg/l	15 mg/l	20 mg/l	30 mg/l	45 mg/l
0	6.87	6.77	6.89	6.57	6.77	6.71
2	6.9	6.78	6.9	6.56	6.76	6.72
4	6.89	6.77	6.9	6.56	6.78	6.72
5	6.9	6.77	6.9	6.51	6.75	6.68
7	6.89	6.74	6.88	6.5	6.73	6.65
9	6.88	6.72	6.86	6.53	6.75	6.7
11	6.91	6.75	6.89	6.55	6.77	6.74
14	6.93	6.77	6.9	6.62	6.82	6.76
17	6.92	6.82	6.93	6.65	6.83	6.79
23	6.9	6.86	6.95	6.71	6.88	6.87
27	6.92	6.88	6.96	6.74	6.88	6.84
31	6.95	6.9	6.97	6.72	6.89	6.86
35	6.97	6.87	6.98	6.75	6.91	6.86
41	7.01	6.89	6.99	6.75	6.91	6.81

Table C2. AGB system ORP value Vs. Injecting Cu^{2+} Conc. (mg/l)

Test Time (Hour)	Cu^{2+} Conc. = 5 mg/l	10 mg/l	15 mg/l	20 mg/l	30 mg/l	45 mg/l
0	-231.9	-239.6	-237.1	-240.4	-232.6	-229.4
2	-232.2	-238.5	-238	-239.5	-232.5	-229.5
4	-233.4	-238.2	-238.2	-238.7	-233.1	-228.5
5	-227	-225.7	-232	-224.6	-225.9	-220.2
7	-229.4	-230.4	-230	-218.5	-221.5	-209.2
9	-232	-234.5	-225.9	-222.6	-220.5	-210.8
11	-233.5	-236.3	-232.1	-224.2	-223	-211.9
14	-235.4	-240.2	-236	-229.7	-225.1	-213.7
17	-236.1	-241.9	-238.1	-231.8	-227.6	-223.5
23	-238.1	-242.1	-241.9	-235.4	-229.7	-230.5
27	-239.4	-244.6	-243.4	-238.4	-230.1	-233.2
31	-241.1	-248.1	-245.1	-239.5	-231.3	-235.9
35	-241.6	-249.9	-245.7	-240.4	-232.6	-238.5
41	-244.8	-251.6	-246.3	-241.9	-233.7	-237.7
47					-235	-239.1
57						-237.3

Table C3. AGB system COD removal efficiency (%) Vs. Injecting Cu^{2+} Conc. (mg/l)

Test Time (Hour)	Cu^{2+} Conc. = 5 mg/l	10 mg/l	15 mg/l	20 mg/l	30 mg/l	45 mg/l
0	88	84.6	86.2	85.1	89	91.4
2	88.9	85	86.8	86.2	88	93.4
4	87.1	85	89.8	88	89.4	91.4
5	87.6	85.7	89.2	88.7	87.1	92.4
7	86.3	86.9	88.6	89.5	87.1	91.9
9	87.1	86.1	80.8	87.6	87.1	90.9
11	86.3	86.1	79	89.5	88	89.9
14	81.6	85.7	77.2	86.6	85.3	85.3
17	78.1	85.4	82.6	85.1	77	80.7
23	80.7	84.6	85	84.4	83.9	78.7
27	84.1	81.6	86.8	83.7	88.5	87.3
31	85	80.4	87.4	86.9	86.2	92.9
35	85.8	82	86.8	85.8	87.6	93.4
41	87.1	84.8	86.8	84.7	83.9	91.9
47					87.6	89.9
57						90.4

Table C4. AGB system pH value Vs. Injecting Phenol Conc. (mg/l)

Time (Hour)	Phenol Conc. =10 mg/l	20 mg/l	30 mg/l	45 mg/l
0	6.95	7.02	7.07	7.07
2	6.94	7.01	7.08	7.05
4	6.94	7.01	7.06	7.05
5	6.93	7	7.04	7.04
7	6.95	6.98	7.02	7.06
9	6.99	6.98	7.01	7.03
11	7.02	6.99	7.03	7.04
14	7.07	7	7	7.03
17	7.09	6.99	7.03	7.05
23	7.07	7	7.01	7.03
27	7.1	7.03	7.03	7.05
31	7.16	7.05	7.02	7.04
35	7.1	7.04	7.03	7.05
41	7.11	7.05	7.02	7.07
51	7.04	6.99	7.07	7.06
63	6.99	6.96	7.1	7.05

Table C5. AGB system ORP value Vs. Injecting Phenol Conc. (mg/l)

Time (Hour)	Phenol Conc. =10 mg/l	20 mg/l	30 mg/l	45 mg/l
0	-244.3	-254.6	-256.1	-248.5
2	-243.7	-254	-256.9	-248.6
4	-243.9	-254.1	-256.4	-248.2
5	-220.9	-228.4	-248.7	-233.1
7	-231.9	-230.1	-240.5	-237.1
9	-238.8	-231.9	-245.3	-240.1
11	-242.3	-231.6	-246.1	-243.8
14	-244.8	-232	-249	-244.5
17	-246.1	-235.4	-250.2	-246.7
23	-251.2	-239.1	-252	-247.1
27	-253.5	-238.3	-252.7	-249.2
31	-255.6	-239.2	-252.8	-248.7
35	-257.4	-242.2	-255	-247.9
41	-256.3	-247.6	-257.1	-248.7
51	-256.7	-252.7	-258.1	-247.6
63	-249.9	-257.3	-259.7	-249.2

Table C6. AGB system COD removal efficiency (%) Vs. Injecting Phenol Conc. (mg/l)

Time (Hour)	Phenol Conc. =10 mg/l	20 mg/l	30 mg/l	45 mg/l
0	80.00	85.99	85.42	90.86
2	79.61	86.41	84.21	89.72
4	81.18	85.14	84.62	90.48
5	81.96	79.62	83.81	86.29
7	78.04	75.37	85.02	86.67
9	69.41	71.55	67.61	76
11	72.16	69.43	60.73	64.58
14	74.90	74.10	68.83	65.72
17	73.73	74.52	77.73	72.19
23	72.94	75.37	81.38	79.43
27	74.51	76.65	84.21	84.38
31	75.69	77.49	84.62	85.91
35	77.25	80.04	84.62	85.14
41	79.61	83.86	85.42	87.05
51	80.78	82.59	84.62	88.57
63	80.39	85.14	85.83	88.95

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