

# **Review of Bioreactor Designs Applicable to Oil Sands Process-Affected Water Treatment**

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## Oil Sands Research and Information Network

The Oil Sands Research and Information Network (OSRIN) is a university-based, independent organization that compiles, interprets and analyses available knowledge about managing the environmental impacts to landscapes and water affected by oil sands mining and gets that knowledge into the hands of those who can use it to drive breakthrough improvements in regulations and practices. OSRIN is a project of the University of Alberta's School of Energy and the Environment (SEE). OSRIN was launched with a start-up grant of \$4.5 million from Alberta Environment and a \$250,000 grant from the Canada School of Energy and Environment Ltd.

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### Citation

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## REPORT SUMMARY

The objectives of our research program were to: (1) study biological activities in oil sands mature fine tailings and oil sands process-affected water, (2) develop microbial biofilm seed to support engineered biological processes with enhancement measures, and (3) review available bioreactor technologies and select bioreactors for continuous operation in the next phase of the study. This report focuses on the literature review. A summary of the results of two M.Sc. theses focusing on objectives 1 and 2 of the research program are provided as an appendix to this report. Further information is available in the theses.

We reviewed 89 papers (from 1980 to 2014) covering eight types of bioreactors with an emphasis on their performance in treating recalcitrant industrial wastewaters. Three types of reactors were selected for further analysis because they have been successfully developed and used for removal of refractory organic compounds from industrial wastewaters. They are moving-bed biofilm reactor, membrane bioreactor, and up-flow anaerobic sludge blanket reactor. The literature review confirmed our initial understanding that in biodegradation of recalcitrant organic compounds, a successful strategy is to first employ an anaerobic bioreactor to break down primarily large molecular organic compounds, increasing their biodegradability, and then use an aerobic bioreactor for the biodegradable organic compounds. Biofilm, or aggregated microbial growth with mixed microbial populations including both anaerobic and aerobic species, is more effective in biodegradation of recalcitrant organic compounds and more resilient to survive in harsh environmental conditions.

Based on the literature search, we have selected moving-bed biofilm reactor as the first reactor type for continuous operation. This type of bioreactor can support biofilm growth, can be operated under both anaerobic and aerobic conditions, has been tested on a variety of wastewaters, and has the advantages of low cost and ease of operation. The bioreactor system has been designed, fabricated, installed and tested. It is ready for continuous operation, pending funding for the next phase of continuous bioreactor operation. The selection of a second type of bioreactor with different configuration and superior performance is in progress.

## **ACKNOWLEDGEMENTS**

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# 1 INTRODUCTION

Tailings ponds contain significant amounts of organic contaminants that cannot be released to the environment without further treatment. Oil sands process-affected water (OSPW) treatment has been a challenge for oil sands companies due to specific influent characteristics, such as recalcitrant organic compounds, toxicity, and high salinity. Reliable, cost-effective and highly-efficient biotechnologies have been successfully applied in the treatment of other industrial wastewaters. These technologies have the potential to be applied for treating OSPW.

The objectives of the research program were to: (1) study biological activities in oil sands mature fine tailings (MFT) and OSPW, (2) develop microbial biofilm seed to support engineered biological processes with enhancement measures, and (3) review available bioreactor technologies and select bioreactors for continuous operation in the next phase of the study. This report focuses on the literature review. A summary of the results of two M.Sc. theses focusing on objectives 1 and 2 of the research program are provided in the [Appendix](#). Further information is available in the theses.

This report presents an overview with critical analysis of 89 papers from 1980 to 2014 related to the technical applicability of biological treatment for OSPW. Particular focus is given to moving-bed biofilm reactors, membrane bioreactors and up-flow anaerobic sludge blanket reactors, which are mostly developed and employed for removal of refractory organic compounds from industrial wastewater. Selected information is presented such as pH, hydraulic retention time, organic loading rate, characteristics of influent and treatment performance.

It is evident from the literature that the selection of the most suitable bioreactor depends on its characteristics, technical applicability and potential constraints, cost-effectiveness, regulatory requirements and long-term environmental impacts. There has been no full-scale bioreactor application for OSPW treatment; however, there has been some progress on bioreactor treatment for commercial naphthenic acid removal. To meet the unique challenges of OSPW, a system that combines aerobic and anaerobic bioreactors may be an effective and sustainable treatment process. The same principles of biological treatment and bioreactor applications are applicable as well for addressing the issue of end-pit lake water remediation.

## 1.1 Oil Sands Context

The Athabasca oil sands deposit, covering more than 75,000 square km, is the third-largest proven oil reserves in the world with approximately 170.4 billion barrels of recoverable bitumen (Kannel and Gan 2012). The Clark Hot Water Extraction Process, a combination of hot water, steam, and caustic (NaOH), is used to separate the bitumen from the oil sands. During this process, a large volume of fresh and recycled water is consumed and large volumes of high water content tailings are produced. The tailings are composed of sand, silt, clay, and a small amount of residual bitumen. The acute and chronic toxicity of fresh OSPW to aquatic biota are

commonly attributed to a complex mixture of organic acids that include naphthenic acids (NAs)<sup>1</sup> (Afzal et al. 2012).

For every unit volume of bitumen recovered, there are 7 to 8 volume units of wet sand and MFT that need to be handled, and 10 volume units of water (recycle and make up) that are pumped around the system (Flint 2005). About 80% to 88% of the water used in the extraction process is recycled (Kannel and Gan 2012). The balance – about 3 cubic metres of water per cubic metre of bitumen – is trapped in the tailings pond and the pores of the sand in beaches and dykes (Flint 2005). At current production rates it is estimated that over 1 billion m<sup>3</sup> of OSPW will be accumulated in the Athabasca area by the year 2025 (Kannel and Gan 2012).

With oil production industry growth in the Athabasca area, concerns about the possible adverse impacts of naphthenic acids in OSPW on aquatic resources are magnified. Environmental management of OSPW is becoming more stringent (Energy Resources Conservation Board 2009) and its treatment is becoming a challenge for researchers, regulators and engineers. In addition to physical and chemical processes, biological processes are an attractive alternative for treatment of OSPW. Where biological processes are feasible, they are almost always more economical in comparison to chemical processes for treatment of large volumes of water containing organic contaminants. For biological treatment, the OSPW-related challenges are toxicity, the low biodegradability, lower temperature and high salt concentration (Allen 2008).

## **2 BIOREACTOR TYPES, CHARACTERISTICS AND PERFORMANCE**

There are some reviews on microbial degradation of NAs in aquatic environments (e.g., Clemente and Fedorak 2005, Headley and McMartin 2004) and on options for in-situ bioremediation of OSPW NAs (Quagraine et al. 2005). Ishak et al. (2012) reviewed biological treatment options for refinery wastewaters and concluded that selection of the appropriate reactor depends on a variety of factors including: cost, available space, and discharge standards. Sutton (2006) provides a chart showing the various types and sub-types of bioreactor configurations.

This report provides a more complete and critical review of recent research on biological treatment of recalcitrant industrial wastewater and the potential types of bioreactors for degrading OSPW pollutants. The bioreactors are assessed in terms of their construction, characteristics and applications and performance is compared to a conventional treatment system (municipal wastewater treatment using activated sludge process).

### **2.1 Aerobic Granular Sludge**

Granular sludge was considered to be a special case of biofilm used in wastewater treatment reactors composed of high density self-immobilized cells. It has a granular appearance, which can withstand a certain amount of pressure. Granular sludge was first found in up-flow

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<sup>1</sup> For more information on tailings pond chemistry see Li, C., A. Singh, N. Klammerth, K. McPhedran, P. Chelme-Ayala, M. Belosevic and M. Gamal El-Din, 2014. Synthesis of Toxicological Behavior of Oil Sands Process-Affected Water Constituents. OSRIN Report No. TR-50. 101 pp. <http://hdl.handle.net/10402/era.39659>

anaerobic sludge blanket (UASB) reactors used to treat industrial wastewater at the end of the 1970s (Lettinga et al. 1980). It is generally thought that the up-flow velocity in a UASB creates a selective pressure to which the organisms have two responses: to be washed out or to stick together and form easily settleable granules.

The formation of aerobic granular sludge was first observed in an aerobic up-flow sludge blanket reactor (Mishima and Nakamura 1991). Morgenroth et al. (1997) found that a sequencing batch reactor (SBR) fed with synthetic wastewater was a good tool to develop aerobic granular sludge after 40 days of incubation (Figure 1). In SBR, the wastewater is mixed with the aerated activated sludge in a pulse-feed mode. Then the sludge and input substrate “react” in a form of batch treatment. This highly dynamic feed regime leads to the growth of stable and dense granules. The formation of granules is favoured when the feeding period time is reduced to some minutes per cycle. Arrojo et al. (2004) have also pointed out that the promotion of slow growing biomass able to store compounds increases the density of the granules formed.

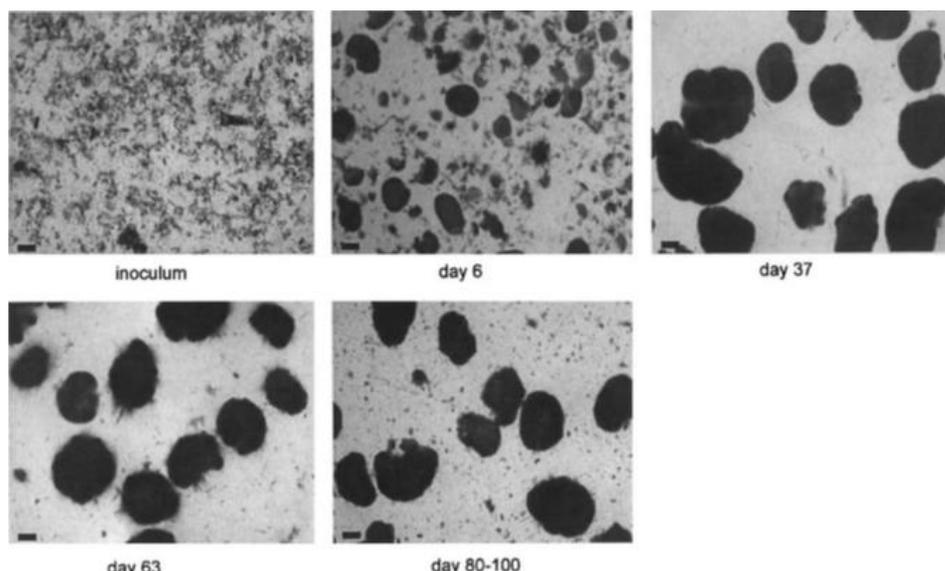


Figure 1. Photographs of sludge in an SBR on different days after inoculation. The length of the short black line on the bottom left of each figure is 1mm (Beun et al. 2002).

Furthermore, high hydrodynamic shear forces seem to stimulate the production of extracellular polysaccharides, as has also been observed in the case of biofilms. For granular sludge, the effect of this factor on the characteristics of the formed granules is not clear. Tay et al. (2002) proposed dependence between shear forces and extracellular polysaccharide production, but the control of hydrodynamics was realized by varying aeration flow rate in the reactors that were used. Thus, different oxygen concentrations were achieved in the reactors operated with different air flow rates, which may influence the formation of the granules. Therefore, SBR has

been demonstrated to be a suitable reactor configuration for aerobic granulation (Liu and Tay 2004).

The advantages of aerobic granular sludge (Adav et al. 2008) are:

1. regular, smooth and nearly round in shape;
2. excellent settleability;
3. dense and strong microbial structure;
4. high biomass retention;
5. ability to withstand high organic loading; and,
6. tolerance to toxicity.

Because of the unique granule attributes, the aerobic granulation technology was recently developed for treating high concentration wastewater containing organics, nitrogen, phosphorus, toxic substances and xenobiotics (Adav et al. 2008). Once the chemical oxygen demand (COD) removal efficiencies were stabilized, Moy et al. (2002) showed the potential of aerobic granules to sustain high organic loading rates by a stepwise increase in organic loading from 6 to 15 kg COD/(m<sup>3</sup>·d) without compromising granule integrity.

Phenol is considered to be toxic to aquatic species (Kibret et al. 2000) and adds an odour to drinking and food-processing water (Rittmann and McCarty 2001). Aerobic granules have been applied to degrade phenol (Chou and Huang 2005, Chou et al. 2004, Jiang et al. 2002, Tay et al. 2005a,b). Tay et al. (2004) demonstrated that the granules degraded phenol at a specific rate exceeding 1 g phenol/(gVSS·d)<sup>2</sup> at 500 mg/L of phenol, or at a reduced rate of 0.53 g phenol/(gVSS·d) at 1,900 mg/L of phenol in an SBR. Removal of 99% of phenol from a saline wastewater with the influent concentration up to 1,000 mg phenol/L and a total cycle time of 17 h was reported by Moussavi et al. (2010).

Sludge granules have been tested for removal of toxic organic compounds. Xie (2003) found aerobic granules to be highly tolerant of toxic heavy metals. Yi et al. (2006) reported on the biodegradation of p-nitro phenol (PNP) by aerobic granules in an SBR. The specific PNP degradation rate increased with corresponding increase in PNP concentration up to 40.1 mg/L, with a peak at 19.3 mg PNP/(gVSS·h), and declined with any further increase in PNP concentration. Wang et al. (2007) efficiently treated wastewater containing 2, 4-dichlorophenol with glucose as a co-substrate. Zhang et al. (2008) noted that methyl t-butyl ether (MTBE) can be efficiently degraded by aerobic granular sludge with ethanol as a co-substrate.

A simultaneous nitrification, denitrification and phosphorus removal process was studied for 450 days in a laboratory-scale SBR under alternate aerobic and anaerobic conditions by Lemaire et al. (2007). Good phosphorus removal and nitrification occurred throughout the SBR with a dominance of *Accumulibacter* spp. (a polyphosphate-accumulating organism, PAO) and

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<sup>2</sup> VSS – Volatile Suspended Solids

*Competibacter* spp. (a glycogen non-polyphosphate-accumulating organism, GAO). *Accumulibacter* spp. was dominant in the outermost 200  $\mu\text{m}$  region of the granule while *Competibacter* spp. dominated in the granule central zone.

The presence of salt in treated effluent was not detrimental to the operation of a reactor once the aerobic granules were formed (Figueroa et al. 2008). Up to 40% ammonia nitrogen was removed via nitrification-denitrification when nitrogen loading rate was at  $0.18 \text{ kg N}/(\text{m}^3 \cdot \text{d})$ .

However, because sludge granulation is a multiple phase process depending on a variety of factors such as composition of the substrate, the operation condition, selective pressure, the shear strength and the sludge settling time, it is difficult to maintain a steady condition for the generation of high density and large size granular sludge. This has restricted industrial practice of the sludge granulation method and most of the studies are laboratory-based.

## 2.2 Sequencing Batch Reactor

The Sequencing Batch Reactor (SBR) is an activated sludge process, but operates in a true batch mode (Figure 2). The essential feature of the SBR system is that each SBR tank carries out functions such as equalization, aeration, and sedimentation in a time sequence, rather than in a space sequence. A properly designed SBR process is a unique combination of equipment and software comprising a complete secondary wastewater treatment facility (Norcross 1992). An SBR can accomplish industrial wastewater treatment, nitrogen and phosphorus nutrient removal, and fully anaerobic treatment.

SBRs in general offer distinct advantages when compared to continuous processes (Kennedy and Lentz 2000), including:

1. high degree of process flexibility in terms of cycle time and sequences;
2. ability to incorporate aerobic and anoxic phases in a single reactor (if desired);
3. sequencing batch closely resembles plug flow operation during the fill cycle;
4. near ideal quiescent settling conditions;
5. no separate clarifiers required; and,
6. elimination of short circuiting.

An SBR system can be also operated as an activated sludge reactor (SBR-AS) or as a biofilm reactor (SBBR) packed with any support material to serve as a substrate for microorganism growth. SBR-AS systems are chosen when extensive growth of activated sludge flocs can be guaranteed. However, in the case of organic compounds with a low bioavailability, when specialized organisms are required, or when a decrease in the BOD/COD ratio of the wastewater is practical, an SBBR system is preferable (Dollerer and Wilderer 1996).

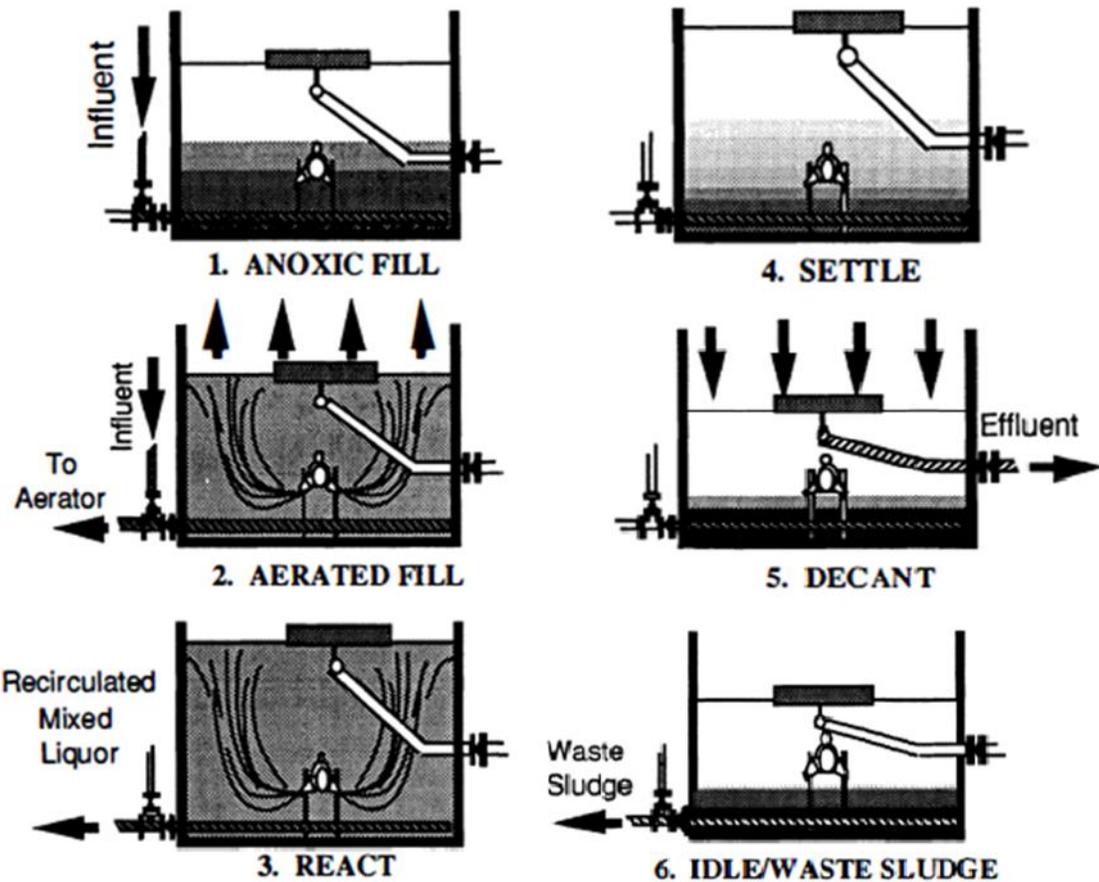


Figure 2. Sequencing Batch Reactor process cycles (Norcross 1992).

SBBR is an attached biomass system working in fill and drawn mode. Its main advantages are:

1. It is possible to carry out in a single operative unit all the phases of an integrated oxidative treatment by inserting a chemical oxidation step (i.e., ozonation) inside the biological treatment (biological → chemical → biological);
2. The selection of microorganisms particularly effective for degrading toxic and/or recalcitrant compounds is favoured; and,
3. It is possible to maintain a uniform biomass concentration along the whole height of the bed, assuring performance stability even under overloading conditions.

Dollerer and Wilderer (1996) conducted bench-scale experiments with two different types of fixed bed reactors to investigate the potential of SBBR technology for treatment of different hazardous waste landfill leachates. An average dissolved organic carbon (DOC) removal rate of 68% was achieved with a 12 h cycle. The emission of biodegradable volatile organic substances was observed to be significantly reduced by using a bubble-free aeration system.

SBBR treatment of industrial wastewater showed comparatively higher efficiency to the corresponding suspended growth and granular activated carbon (GAC) configured systems (Mohan et al. 2007). Fongsatitkul et al. (2008) investigated the ability of an SBBR system to treat four industrial wastewaters: textile, landfill leachate, seafood and slaughterhouse effluents. During this study, all four wastewaters experienced COD and total Kjeldahl nitrogen (TKN) removals greater than 81%, while the total phosphorous (TP) removals were lower, ranging from 57% to 94%.

An innovative process based on ozone-enhanced biological degradation, carried out in an aerobic granular biomass system (SBBGR – Sequencing Batch Biofilter Granular Reactor), was tested at pilot scale for tannery wastewater treatment. Tannery wastewater was chosen as representative of industrial recalcitrant wastewater. The SBBGR technology was successful in removing 80% to 90% of the COD, suspended solids and ammonia content up to organic loading values of 3.5 kg COD/(m<sup>3</sup>·d) (Di Iaconi et al. 2008). In comparison with the conventional treatment, the SBBGR was able to reduce sludge production by 25 to 30 times and to save 60% of operating costs (Di Iaconi et al. 2010).

Moussavi et al. (2010) conducted several tests to assess an aerobic granular sequencing batch reactor's (GSBR) performance for degradation and COD removal of phenol as the sole substrate from saline wastewater. The results showed that the reactor could remove more than 99% of phenol from the feed saline wastewater at inlet phenol concentrations of up to 1,000 mg/L and total dissolved solid (TDS) concentrations up to 8%. The total cycle time of this system was 17 h, including 15.5 h aerating, 1 h filling, and 30 min settling, decanting and idle.

When a GSBR was applied for treating municipal wastewater, the system achieved removal efficiencies of 80% to 90% for COD, total suspended solids and ammonia regardless of the hydraulic residence time investigated (i.e., from 12 to 4 h).

Choi and Liu (2014) constructed two SBRs and filled them with two different inocula of activated sludge and mature fine tailings to treat OSPW. When 100% raw OSPW was fed into these reactors, COD removal reached 12% and 20% in the AS-SBR and the MFT-SBR, respectively. Maximum removal of acid-extractable organics was 8.7% and 16.6% in the AS-SBR and the MFT-SBR, respectively, with an HRT of one day.

SBR technology has proven to be highly adaptable for some specialized applications by simply altering volumetric exchange rates, cycle times, sequence of phases and sludge age. It has become a worldwide accepted alternative to the continuous flow activated sludge process (Moletta and Wilderer 2001).

### **2.3 Moving-Bed Biofilm Reactor**

The moving-bed biofilm reactor (MBBR) is a highly effective biological treatment process that was developed on the basis of conventional activated sludge processes and fluidized-bed reactors. It is a completely mixed and continuously operated biofilm reactor, where the biomass is grown on small carrier elements that have a little lighter density than water and are kept in movement along with a water stream inside the reactor. The fluidization inside a reactor can be

caused by aeration in an aerobic reactor and by a mechanical stirrer in an anaerobic or anoxic reactor (Borkar et al. 2013).

The moving bed biofilm reactor has many advantages when compared with conventional biological treatment systems: high sludge age, no sludge recycling, low head-loss, use of the whole tank volume for biomass growth, less space, no influence of biomass separation, operational stability and improved mass transfer. Therefore, MBBRs can handle larger organic loading than conventional biological treatments.

Rusten et al. (1999) tested MBBRs at organic loads up to  $53 \text{ g BOD}_5/(\text{m}^3 \cdot \text{d})$  and they always removed the easily biodegradable biological oxygen demand (BOD) fraction, ranging from about 60% to 80%. At organic loads from 10 to  $20 \text{ g BOD}_5/(\text{m}^3 \cdot \text{d})$ , slowly biodegradable organic matter was also metabolized, sometimes removing more than 95%  $\text{BOD}_5$ . After polishing in an activated sludge unit, the final effluent had an average concentration of only  $3.4 \text{ mg filtered BOD}_5/\text{L}$ .

With a very high specific biofilm surface area, MBBRs followed by activated sludge offer a compact process combination for complete biological treatment of chemical industry wastewaters. Hosseini and Borghei (2005) operated two MBBR units simultaneously at different hydraulic retention times (HRT) of 24, 20, 16, 12 and 8 h with phenol concentrations of 200, 400, 620 and  $800 \text{ mg/L}$ . Throughout the experiments the ratio of phenolic COD concentration to total COD was changed from 0.2 to 1. At a ratio of 0.6, maximum COD removal efficiency (96%) was observed, and this ratio was effective at all HRTs (Hosseini and Borghei 2005).

Delnavaz et al. (2008) used light expanded clay aggregate as carriers and operated an MBBR in an aerobic batch and continuous conditions. Evaluation of the reactors' efficiency was assessed varying retention times and influent COD levels (filling ratio of 50%). After three days operation, the maximum obtained removal efficiencies were 90% (influent COD =  $2,000 \text{ mg/L}$ ), 87% (influent COD =  $1,000 \text{ mg/L}$ ) and 75% (influent COD =  $750 \text{ mg/L}$ ) for aniline, para-diaminobenzene and para-diaminophenol, respectively. If the filling ratio decreased from 50% to 30%, removal rate decreased 6% for both para-diaminobenzene and para-aminophenol and increased 7% for aniline.

MBBRs are easily alternated between aerobic and anaerobic conditions, which can achieve nitrification, BOD/COD removal and phosphorous removal (McQuarrie and Boltz 2011). Li et al. (2011b) used a laboratory-scale MBBR with a volume of 4 L to study the biodegradation of coal gasification wastewater. Maximum removal efficiencies of 81%, 89%, 94% and 93% were obtained for COD, phenols,  $\text{SCN}^-$  and  $\text{NH}_4^+-\text{N}$ , respectively (Li et al. 2011b).

Because of the biofilm diffusion limit, MBBRs exhibit greater resistance to the adverse chemical conditions presented by industrial wastewaters than conventional biological treatment. MBBRs can supply a good microenvironment for specialized microbes to digest toxic organic compounds including aromatic compounds, organic chlorides, and other contaminants. Bassin et al. (2011) found that nitrification of treated domestic sewage was higher than 90% for all tested chloride concentration up to  $8,000 \text{ mg/L}$  in a bench-scale MBBR. MBBRs can provide adequate

conditions for adaptation of nitrifying microorganisms even under stressing and inhibitory conditions.

Moussavi et al. (2009) tested the performance of a moving-bed sequencing batch reactor (MSBR) to remove phenol from wastewater. The optimum hydraulic residence time for the MSBR was 40 h and the critical phenol loading rate was 83.4 g phenol/(m<sup>3</sup>·h), which resulted in a phenol removal rate of 99%.

Aerobic MBBRs act as a COD-polishing and ammonium removal step, and anaerobic MBBRs play a major role in COD removal due to methanogenesis. In aerobic conditions, nitrification inside the MBBR can be enhanced by the addition of nitrifying sludge into the reactor (Li et al. 2011a). Chen et al. (2008b) reported that the contribution of an anaerobic MBBR to total COD removal efficiency reached 91% at an organic loading rate (OLR) of 4.08 kg COD/(m<sup>3</sup>·d), and gradually decreased to 86% when feed OLR was increased to 15.70 kg COD/(m<sup>3</sup>·d). Because of the additional contribution of the aerobic reactor, the total COD removal efficiency of the system had only a slight decrease from 94% to 92% even though the feed OLR was increased from 4.08 to 15.70 kg COD/(m<sup>3</sup>·d). A decrease in COD removal efficiency of only 7% was observed when the OLR was increased by four times within 24 h, and the system could recover the original removal efficiency in 3 days. Therefore, an MBBR process train with an anaerobic-aerobic arrangement is a possible way to achieve removal of COD and ammonium simultaneously.

Schneider et al. (2011) tested an MBBR on oil refinery wastewater and achieved the best performance of 89% COD removal and 86% N-NH<sub>4</sub><sup>+</sup> removal at an HRT of 6 h. After ozonation in series with a biological activated carbon column, the effluent met the requirements for water reuse in the oil refinery.

MBBR is a promising technology for use in OSPW treatment, and has been found to be a commercially successful process. There are presently several hundred small and on-site treatment units in Germany and more than 400 full scale wastewater treatment plants in operation in 22 different countries (Delnavaz et al. 2008).

## 2.4 Membrane Bioreactor

Membrane Bioreactor (MBR) technology combines the biological degradation process (activated sludge process) with a direct solid-liquid separation (membrane filtration process). By using micro or ultra-filtration membrane technology (with pore sizes ranging from 0.1 to 0.4 μm), MBR systems can completely retain bacterial flocs and virtually all suspended solids within the bioreactor. The quality of water treated by MBR systems is equal to the combination of secondary clarification and effluent microfiltration. As a result, the MBR process has now become an attractive alternative for the treatment and reuse of industrial and municipal wastewaters, as evidenced by the constantly rising numbers and capacity of MBR systems. Lin et al. (2012) and Mutamin et al. (2012, 2013) provide reviews of membrane bioreactor applications, limitations and performance.

Generally, an MBR system consists of an aeration tank and a membrane module (Figure 3). The first generation of MBR systems used in the 1980s was mainly based on the side-stream

configuration, for which the membrane is located outside the bioreactor and the biomass is circulated at high cross-flow velocity (usually around 2 to 4 m/s). At that time, tubular membranes remained the norm in the industry, and the permeation was operated from inside to outside the tubes (i.e., 'in-to-out' filtration). However, the external configuration limited wider application in treatment of municipal wastewater in North America because of high power consumption. After the mid-1990s, with the development of submerged systems, MBR applications in municipal wastewater expanded. In the past 10 years, MBR technology has been of increased interest both for municipal and industrial wastewater treatment in North America (Yang et al. 2006).

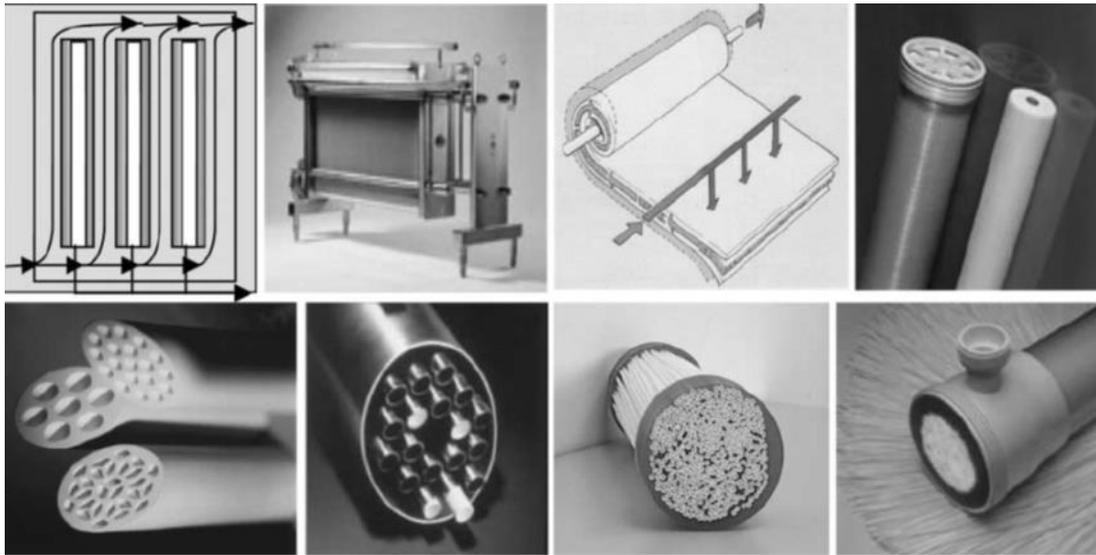


Figure 3. Membrane modules.

From left to right, top row: plate and frame module schematic, plate and frame module, spiral wound module schematic, spiral wound module

Bottom row: monoliths, tubular membranes, capillary membranes, hollow fibers (Vankelecom 2005).

Characteristics of MBRs are:

1. High biomass concentration: biomass concentrations up to 35 g/L are feasible, which allow this kind of reactor to handle high organic loading rates during operation;
2. High oxygen consumption: due to high biomass concentrations, high minimum maintenance energy is needed, in addition to energy for biosynthesis and cell growth. Consequently, high oxygen concentrations are required in aerobic membrane systems to ensure continuous biosynthesis and cell growth;
3. Good effluent quality: as a result of membrane separation, solids retention time is independent of hydraulic retention time. The long solids retention time has the

potential to allow low growth rate bacteria to survive in the reactor which is important for OSPW treatment given its complex chemical and microbiological characteristics. Therefore, MBR is the most attractive option for situations where long solids retention times (SRT) are necessary to achieve the removal of pollutants; and,

4. Low sludge production: sludge production is much lower than in conventional aerobic systems due to the high temperatures and the relatively low food to microorganism (F/M) ratio, which reduce the operating cost.

Based on the above analysis, membrane bioreactor processes are well suited for applications that require a small footprint. Application of membrane bioreactor technology makes it possible to recover valuable components (such as phosphate from the sludge) from effluent streams, reuse contaminated process water, and degrade some recalcitrant organic pollutants. Food, pharmaceutical, paper and pulp, landfill, textile and meat industries are some of the examples for which MBR has been successfully applied to treat high-strength wastewaters (Yang et al. 2006). An estimated 500 commissioned MBR plants, each treating more than 20 m<sup>3</sup>/d of industrial wastewater, were reported operating in Europe as of 2008 (Le-Clech 2010).

Llop et al. (2009) found that when MBR was used for petrochemical wastewater, the reduction in COD and total organic carbon (TOC) was high. The MBR treatment of olefin process wastewater reduced COD and TOC by around 90% with pH at 6 and 9, and more than 90% of the suspended solids (SS) were removed.

A full-scale MBR system treating livestock wastewater was reported by Kim et al. (2005). Based on 6 months operation data, BOD and SS removal were about 99.9% and COD, TN and TP removal were 92.0%, 98.3% and 82.7%, respectively.

Scholzy and Fuchs (2000) operated an MBR for treating oil contaminated water. The removal rate of oil, surfactants and COD were 99.99%, 98%, and 93% to 95%, respectively, which shows MBR has good potential application for process wastewater recycling purposes in industry.

Phenol can reduce the biological degradation of other wastewater components. Barrios-Martinez et al. (2006) demonstrated that MBR treatment was effective in treating phenol-containing effluent. Experimental results by Marrot et al. (2006) showed that it was possible to treat effluents containing high phenol concentrations by activated sludge at typical biomass concentrations in a membrane bioreactor. Phenol biodegradation by mixed culture was studied in an MBR over a period of 285 days (Marrot et al. 2008). The MBR, with acclimatized activated sludge, achieved significant phenol degradation (95% average COD removal efficiency and greater than 99% phenol removal efficiency) without supplemental reagent addition. Excellent effluent quality was obtained regardless of the extremely short SRT (5 to 17 days). Ersu and Ong (2008) tested the performance of a membrane bioreactor with a tubular ceramic membrane for phenol removal under varying hydraulic retention times and a fixed sludge residence time of 30 days. They showed that the MBR could be operated safely without upsets for concentrations up to 600 mg/L of phenol at 2 to 4 hours HRT and 30 days solids retention time (Ersu and Ong 2008).

Even for high salinity wastewater, MBR has exhibited capability for phenol removal. Dosta et al. (2011) operated two MBRs with submerged flat membranes, one at lab-scale and the other at pilot-plant scale, at ambient temperature to treat an industrial wastewater characterized by low phenol concentrations (8 to 16 mg/L) and high salinity (150 to 160 mS/cm). During the operation of both reactors, the phenol loading rate was progressively increased and less than 1 mg phenol/L was detected in the effluent even at very low HRTs (0.5 to 0.7 days).

Le-Clech (2010) has noted several problems that may arise during operation of MBRs:

1. Pre-treatment and clogging: MBR influent requires proper and efficient pre-screening (for removal of large solid waste in the wastewater to prevent the membrane module from clogging);
2. Fouling and fouling control: one of the main drawbacks of MBR remains the unwanted deposition of materials on the membrane surface during filtration. This fouling phenomenon has been studied by many groups and recent literature reviews have summarized the current understanding and advancements in this field (Drews 2010, Le-Clech et al. 2006);
3. Aeration and oxygen transfer: oxygen is required in MBR processes to maintain the existing biomass and to degrade the biodegradable pollutants and other nitrogen-based compounds. The floc size and concentration are two of the main factors influencing the quantity of oxygen which can be transferred from liquid phase into biofilm for the biological degradation; and,
4. Energy consumption and cost considerations: although the cost of membrane modules keeps decreasing, the capital investment for building an MBR plant remains higher than for a conventional treatment system. Due to the high energy demand, the MBR technology also has greater maintenance and operation cost, when compared to conventional techniques. Cost is a key limitation for large-scale MBR application.

## **2.5 Up-Flow Anaerobic Sludge Blanket Reactor**

The up-flow anaerobic sludge blanket (UASB) reactor (Figure 4) is an advanced anaerobic treatment that is characterized by an anaerobic granular sludge with a notably high metabolic activity and good bio-solids settling ability, which was developed by Dr. Gatzke Lettinga and colleagues in the late 1970s at Wageningen University, The Netherlands.

From a hardware perspective, a UASB reactor is at first appearance nothing more than an empty tank. Wastewater enters the tank at the bottom of the reactor, then passes upwards through an anaerobic sludge bed where the microorganisms in the sludge come into contact with wastewater-substrates. The resulting anaerobic degradation process typically is responsible for the production of gas (e.g., biogas containing CH<sub>4</sub> and CO<sub>2</sub>). The upward motion of released gas bubbles causes hydraulic turbulence that provides reactor mixing without any mechanical parts. At the top of the reactor, the water phase is separated from sludge solids and gas in a three-phase separator (also known the gas-liquid-solids separator). Baffles are used to deflect gas to the gas-

cap opening. These characteristics allow the reactor to maintain a high bio-solid content while operating at relatively low HRT.

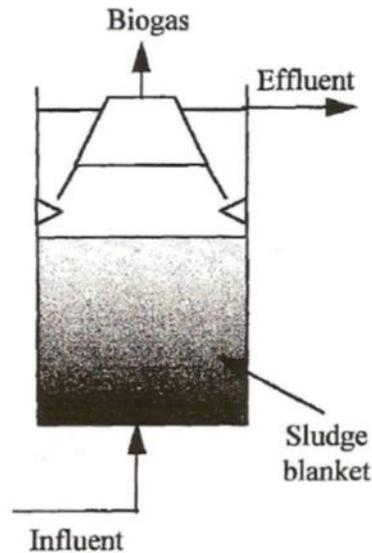


Figure 4. The UASB reactor concept (Christensen et al. 1984).

Granulation in UASB reactors is important in the treatment of various industrial wastewaters containing toxic substance due to their compact structure which protects the bacteria from inhibitory and toxic pollutants. Another important feature of the UASB design is the gas-solids separator which provides a quiescent zone in the upper part of the reactor, where suspended solids (active biomass) can settle. This facilitates the movement of sludge back into the reactor bed. The anaerobic sludge obtained in this way maintains superior settling qualities if chemical and physical conditions favourable to sludge flocculation and maintenance are provided. The development of a highly settleable, pelletized and active sludge is a main advantage of the UASB concept.

Problems with UASB reactors are usually associated with the inability of sludge to develop suitable granules. With certain types of waste, a granular sludge will develop quite readily, while with other wastes this will happen only very slowly or not at all (Heffeman et al. 2011, Sigge and Britz 2007, Veeresh et al. 2005).

Development of granular sludge depends on a number of factors, including: the characteristics of the substrate, the inoculum used during start-up, concentration of divalent cations, the liquid velocity and feed concentration, compression of the sludge bed, rate of gas production and the presence of inert particles (Del Nery et al. 2008). Sponza (2001) found massive initial granules were developed 1.5 months after start-up, which grew at an accelerated pace for 7 months and then became fully grown. The effect of operational parameters such as influent tetrachloroethylene (TCE) concentrations, COD and TCE loading, food to microorganism (F/M)

ratio and specific methanogenic activity (SMA) were also considered during granulation. The maximum diameter of the cultivated granular sludge was 2.5 mm with an SMA of 1.32 g COD/(gTSS·d). COD and TCE removal efficiencies of 92% and 88% were obtained when TCE and COD loading rates were 30 mg COD/(L·d) and 10.5 mg COD/(L·d), respectively.

A high sludge concentration can be retained inside a UASB reactor with simple and low cost equipment. This high sludge concentration allows the operation of the reactors under high organic loads. Singh et al. (1996) described the feasibility of anaerobic treatment of a low-strength (500 mg COD/L) synthetic wastewater using a semi-pilot-scale UASB reactor under ambient temperature conditions (20 to 35 °C); with an HRT of 3 h and corresponding organic loading of 4 kg COD/(m<sup>3</sup>·d), 90% to 92% COD and 94% to 96% BOD reductions were achieved.

Fang et al. (1995) found that a UASB consistently removed 97% to 99% of COD from wastewater containing concentrated mixed volatile fatty acids (VFA) at 37 °C at loading rates of up to 24 g COD/(L·d), corresponding to a F/M of 0.78 g COD/(gVSS·d). Li et al. (1995) found that a UASB removed 97% to 99% of soluble COD from wastewater containing concentrated benzoate at 37 °C, pH 7.5, an HRT of 9.8 h, and loading rates up to 30.6 g COD/(L·d) based on the reactor volume.

Due to the incorporation in the granules of a balanced syntrophic consortia, and to the improvement of the interspecies metabolic transfer rate, the degradation kinetics in a UASB are enhanced. Li et al. (1995) found that about 95.2% of the total COD removed was converted to methane; 0.034 g of volatile suspended solids was produced for each gram of COD removed. Therefore, the performance of UASBs is good even with high organic loading rate.

In a UASB, the structural characteristics of bacterial aggregates and high biomass retention increase the tolerance of anaerobic bacteria to toxic compounds. For example, Fang et al. (1996) demonstrated that phenol in wastewater could be effectively degraded in a UASB reactor. With a 1:1 effluent recycle ratio, over 97% of phenol was removed at 37 °C and pH 6.9 to 7.5 with 12 h hydraulic retention time for phenol concentrations up to 1,260 mg/L; which correspond to 3,000 mg/L of COD and a loading rate of 6 g COD/(L·d) (Fang et al. 1996). Ke et al. (2004) treated a synthetic wastewater containing phenol as the sole substrate in a 2.8 L UASB reactor at ambient temperature. The reactor was able to remove 99% of phenol up to 1,226 mg/L in wastewater with an HRT of 24 h. For HRTs below 24 h, phenol degradation efficiency decreased with HRT, from 95.4% at 16 h to 93.8% at 12 h. It further deteriorated to 88.5% when HRT reached 8 h (Ke et al. 2004).

UASBs are also capable of treating some pesticides if the granular sludge forms properly. Erguder et al. (2003) tested a lab-scale, two-stage continuous UASB reactor system fed with ethanol as the sole carbon source and indicated that anaerobic granular cultures could be successfully acclimated to dieldrin (DLD). COD removal rate of the two-stage system was in the range of 88% to 92%. The influent DLD concentration of 10 mg/L was reduced by 44% to 86% and 86% to 94% in the second stage and overall UASB system, respectively.

Rincon et al. (2003) reported on the anaerobic treatment of three different produced waters from the extraction of light, medium and heavy crude oil using lab-scale UASB reactors. Granular

sludge from a UASB reactor treating brewery wastewater was used as the inoculum. COD removal in a UASB fed with water separated from extracted light crude was high, with an average 87%. However, the results with UASB reactors fed with water separated from extracted medium and heavy crude oil showed COD removal rate was low – 20% and 37%, respectively. It was assumed that the remaining COD was the sum of non-biodegradable and very slowly biodegradable organic matter in the water because continued operation of the UASB reactor did not bring any improvements. Vieira et al. (2005) investigated the anaerobic biodegradability of produced water from an offshore oilfield in a lab batch reactor. The influent water had 7.6% TDS and 4,700 mg/L COD. After an incubation of 6 to 15 days, reductions of about 57% COD, 44% to 78% total phenols and 42% to 62% oil and grease were achieved.

## 2.6 Expanded Granular Sludge Bed Reactor

A modification of the UASB reactor is the Expanded Granular Sludge Bed (EGSB), which was developed to improve the wastewater-biomass contact during anaerobic treatment by expanding the sludge bed and intensifying the hydraulic mixing. Expanded bed refers to a bed in which the biomass grows on the surface of small particles (typical diameters in the range of 0.2 to 2.0 mm), allowing expansion (fluidization) of the bed. Figure 5 shows the structure of an EGSB.

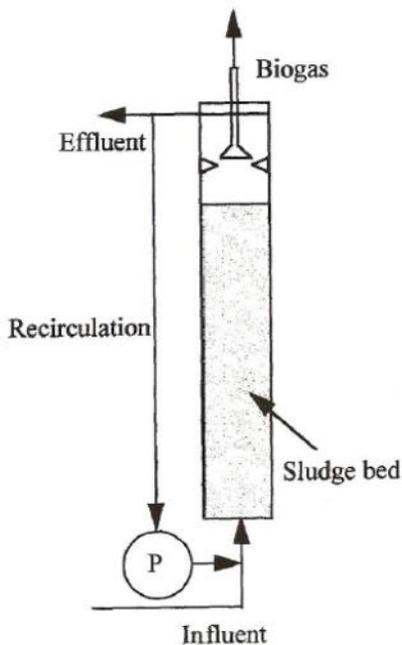


Figure 5. Schematic diagram of an EGSB reactor (Kato et al. 1997).

An EGSB reactor is designed with a height to width ratio ranging from 4:1 to 5:1 to provide the microorganisms in the sludge bed more chance to react with the wastewater (shallow reactors can also be used). This enables the reactor to treat high-strength organic wastewater at a loading rate of about 30 kg/(m<sup>3</sup>·d) which is at least twice the capacity of a UASB (Lim 2006). With the

use of effluent recirculation, liquid upward velocities exceeding 5 to 6 m/h can be achieved, which is significantly higher than the 0.5 to 1.5 m/h range generally applied for UASB reactors (Rittmann and McCarty 2001).

The utilization of tall reactors, or installation of an effluent recycle, or both, increase the up-flow velocity. These factors make the main structure of an EGSB different from that of a UASB. Several full-scale EGSBs have been applied since 1984. Before that, expanded granular sludge bed reactors were only tested at the laboratory/pilot-scale (Heijnen et al. 1989). Kato et al. (1997) confirmed that dissolved oxygen does not have any detrimental effect on the reactor treatment performance, which will make the control of EGSBs easier than that of a UASB.

EGSB reactors can be used to treat low-strength soluble wastewater and wastewater that contains non-biodegradable suspended solids, especially at low to mid temperature (Lim 2006). In EGSB reactors, efficiencies were above 80% at OLRs up to 12 g COD/(L·d) with COD as low as 100 to 200 mg/L (Yoochatchaval et al. 2008).

Collins et al. (1998), showed that an anaerobic expanded bed reactor (AEBR) performed well over a wide range of influent CODs and temperatures, especially under severe conditions (5 °C and 50 mg/L influent COD). The most efficient treatment was obtained with HRTs of 3 to 6 h and influent COD of approximately 150 mg/L.

Collins et al. (2005) operated an expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactor at 15 °C for the treatment of 2, 4, 6-trichlorophenol (TCP)-containing VFA-based wastewaters. The withdrawal and subsequent application of stepwise increments to the TCP loading resulted in steady COD removal.

Sulphide concentration did not have much impact on the performance of EGSBs. Although unable to initiate development of stable granules in synthetic high-sulphide wastewater, Chen et al. (2008a) successfully placed seed sludge from an anaerobic wastewater treatment plant into an EGSB and reported that the EGSB could convert sulphide, nitrate, and acetate simultaneously to  $S^0$ , N-containing gases and  $CO_2$  at loading rates of 3.0 kg S/(m<sup>3</sup>·d), 1.45 kg N/(m<sup>3</sup>·d), and 2.77 kg C/(m<sup>3</sup>·d), respectively, even when sulphide concentration was up to 800 mg/L. An EGSB reactor incubated with bio-granules could simultaneously convert 4.8 kg S/(m<sup>3</sup>·d) of sulphide at 97% efficiency; 2.6 kg N/(m<sup>3</sup>·d) of nitrate at 92% efficiency; and 2.7 kg C/(m<sup>3</sup>·d) acetate at 95% efficiency (Chen et al. 2009).

A full-scale expanded bed reactor (160 m<sup>3</sup>) with overlaid anaerobic and aerobic zones was used for municipal wastewater treatment (Mendonca et al. 2006). In the anaerobic condition, after inoculation and 60 days of operation, the reactor treating 3.40 kg COD/(m<sup>3</sup>·d), at an HRT of 2.69 h, reached mean removal efficiencies of 76% for BOD, 72% for COD, and 80% for TSS, when the effluent had mean values of 225 mg/L of COD, 98 mg/L of BOD and 35 mg/L of TSS. Under these conditions, for nitrogen loading of 0.27 kg N/(m<sup>3</sup>·d), the reactor generated an effluent with mean N-organic of 8 mg/L and N-ammonium of 37 mg/L, demonstrating high potential for ammonification. For the anaerobic-aerobic condition (118th day) the system was operated with an HRT of 5.38 h; average removal efficiencies of 84%, 79%, 76% and 30% were obtained for BOD, COD, TSS, and TKN, respectively (Mendonca et al. 2006).

There are three issues or limitations of the EGSB:

1. Flocculent sludge is washed-out of the reactor. Due to a high liquid up-flow velocity used to improve the mixing regime in EGSBs a higher sludge washout may result. Therefore, a balance must be found with respect to the liquid up-flow velocity needed for sufficient mixing and that for maintaining the sludge at a high level. While high treatment efficiency due to the good wastewater-biomass contact was demonstrated, the required sludge retention levels may represent an obstacle for the feasibility of the EGSB system.
2. No good removal of suspended solids and colloidal matter can be achieved. Soluble pollutants are efficiently treated in EGSB reactors but suspended solids are not substantially removed from the wastewater stream due to the high up-flow velocities applied. Based on the case study of EGSB reactor applications, it is known that the average concentration of suspended solid in the effluent is 75 mg/L, which is more than that specified in the *Wastewater System Effluent Regulations*<sup>3</sup> in Canada (25 mg/L). Therefore post-treatment of EGSB effluent is required which will increase the investment of the wastewater treatment plant.
3. Long time needed for reactor start-up. Sludge granulation is complex and affected by many factors, and is not clearly understood yet. Most microorganisms in granules are denitrifying, nitrifying, acidogenic, and methanogenic bacteria. However, several factors determine characteristics of granules, including: characteristics of organisms, growth rate of organisms, and death rate and decay rate of the organisms (Lettinga 1995). So gradually increasing inlet flow rate and organic load is needed; seeding of granular sludge from other operating reactor is highly recommended.

### 3 CONCLUSIONS AND RECOMMENDATIONS

The literature clearly shows the effectiveness of bioreactor systems in domestic and industrial wastewater treatment, especially for certain kinds of petrochemical materials, such as phenol, benzene and BTEX. The significant contributions of anaerobic bioreactors in the biological systems' overall performance emphasize the importance of reduction in energy consumption, excess sludge production and high tolerance to varying influent water quality compared to conventional aerobic bioreactors.

Most of the bioreactors presented in this work lack large-scale implementation results so further work is required to evaluate the performance of these promising systems on a larger scale, and on OSPW. Based on the literature search, we have selected the moving-bed biofilm reactor for continuous bioreactor operation. This type of bioreactor can support biofilm growth, can be operated under both anaerobic and aerobic conditions, has been tested on a variety of wastewaters, and has the advantages of low cost and ease of operation. This bioreactor system has been designed, fabricated, installed and tested. It is ready for continuous operation, pending

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<sup>3</sup> See <http://laws-lois.justice.gc.ca/eng/regulations/sor-2012-139/FullText.html>

funding for the next phase of continuous bioreactor operation. The selection of a second type of bioreactor with different configuration and superior performance is in progress. In addition, further research and development on the use of combined bioreactor systems is necessary to overcome challenges posed by individual systems.

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## **5 GLOSSARY**

### **5.1 Terms**

#### **Activated Sludge**

A process for treating sewage and industrial wastewaters using air and a biological floc composed of bacteria and protozoa.

#### **Aerobic**

Means “requiring air”, where “air” usually means oxygen.

#### **Anaerobic**

Means “living without air” (as opposed to aerobic).

#### **Anoxic**

Total deprivation of oxygen.

#### **Biofilm**

Any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

#### **Bioreactor**

Refers to any manufactured or engineered device or system that supports a biologically active environment.

### **Chemical Oxygen Demand (COD)**

The COD test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water or wastewater, making COD a useful measure of water quality. It is expressed in milligrams per litre (mg/L), which indicates the mass of oxygen consumed per litre of solution.

### **Copy Number**

The number of plasmid or other DNA molecules in a cell.

### **Hydraulic Retention Time**

A measure of the average length of time that a soluble compound remains in a constructed bioreactor.

### **Influent**

Water, wastewater or other liquid flowing into a reservoir, basin or treatment plant.

### **Sludge**

Solids removed from wastewater during treatment.

### **Solids Retention Time**

The average time the activated sludge solids are in the system. It is an important design and operating parameter for the activated sludge process and is usually expressed in days.

## **5.2 Acronyms**

AAAO	Acetic Acid Amended OSPW (bioreactor)
AEBR	Anaerobic Expanded Bed Reactor
BOD	Biological Oxygen Demand
BOD <sub>5</sub>	Biological Oxygen Demand for 5 days
COD	Chemical Oxygen Demand
DGGE	Denaturing Gradient Gel Electrophoresis
EGSB	Expanded Granular Sludge Bed Reactor
EPS	Extracellular Polymeric Substance
F/M	Food to Microorganism (ratio)
GAC	Granular Activated Carbon
GAO	Glycogen non-polyphosphate-Accumulating Organism
GSBR	Granular Sequencing Batch Reactor
HiPO <sub>x</sub>	High Pressure Oxidation

HPLC-MS	High Pressure Liquid Chromatography – Mass Spectrometry
HTO	HiPOx Treated OSPW (bioreactor)
MBBR	Moving-Bed Biofilm Reactor
MBR	Membrane Bioreactor
MFT	Mature Fine Tailings
OCR	Organic Loading Rate
OSPW	Oil Sands Process-Affected Water
OSRIN	Oil Sands Research and Information Network
PAO	Polyphosphate-Accumulating Organism
SBBGR	Sequencing Batch Biofilter Granular Reactor
SBBR	Sequencing Batch Biofilm Reactor
SBR	Sequencing Batch Reactor
SBR-AS	Sequencing Batch Reactor – Activated Sludge
SEE	School of Energy and the Environment
SMA	Specific Methanogenic Activity
SRB	Sulfate Reducing Bacteria
SRT	Solids Retention Time
SS	Suspended Solids
TDS	Total Dissolved Solids
UASB	Up-flow Anaerobic Sludge Blanket
VSS	Volatile Suspended Solid

### **5.3 Chemicals**

BTEX	Benzene, Toluene, Ethylbenzene and Xylene
C	Carbon
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon Dioxide
DLD	Dieldrin
DO	Dissolved Oxygen
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide

MTBE	Methyl t-Butyl Ether
N	Nitrogen
NA(s)	Naphthenic Acid(s)
NO <sub>3</sub>	Nitrate
O <sub>3</sub>	Ozone
PNP	p-Nitro Phenol
S	Sulphur
S <sup>0</sup>	Elemental Sulphur
SCN <sup>-</sup>	Thiocyanate
SO <sub>4</sub> <sup>2-</sup>	Sulphate
TCE	Tetrachloroethylene
TCP	2, 4, 6-Trichlorophenol
TKN	Total Kjeldhal Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
VFA	Volatile Fatty Acids

## **APPENDIX 1: Laboratory Studies Investigating Microbiological Degradation of Oil Sands Process-Affected Water in Bioreactors.**

At the start of this work, it was hypothesized that indigenous microorganisms from mature fine tailings (MFT) would biodegrade hydrocarbons. This hypothesis was based on the fact that the bacterial consortium is highly adapted to its environment which is rich in hydrocarbons, naphthenic acids (NA) and sulfate. It was also hypothesized that advanced oxidation processes would reduce NA concentrations, and change the composition of the remaining organic matter to predominantly short alkanes, which can be further biodegraded by native bacteria.

Using bioreactors inoculated with bacteria indigenous to MFT to treat oil sands process-affected water presented an opportunity to investigate the hypothesis in a laboratory setting, and to address the question of whether this kind of treatment offers an alternative solution to remediate oil sands process-affected water.

Two M.Sc. theses were prepared to address the first two objectives of the research program:

1. Study biological activities in oil sands MFT and OSPW
2. Develop microbial biofilm seed to support engineered biological processes with enhancement measures

Edited versions of the abstracts and conclusions of each thesis are presented here.

Yu, M., 2014. Biodegradation of organic compounds in OSPW with microbial community indigenous to MFT. University of Alberta, Department of Civil and Environmental Engineering, Edmonton, Alberta. M.Sc. Thesis. 116 pp.

### **Abstract**

Tailings ponds contain significant amounts of organic contaminants that cannot be released to the environment without further treatment. The use of mature fine tailings (MFT) was proposed as a potential source of microorganisms for biological treatment to remove dissolved organic compounds from oil sands process-affected water (OSPW).

To test the capacity of microorganisms indigenous to MFT for removal of organic compounds in OSPW, and to determine whether they could be extracted from MFT to form biofilm on biofilm carriers, two groups of batch bioreactors were established: one treating acetic-acid-supplemented OSPW, and one treating high pressure oxidation (HiPOx)-treated OSPW. In addition, several bioreactors that contained no MFT but MFT-originated biofilm were set up to test the feasibility of using MFT-originated biofilm to biodegrade organic compounds.

The bioreactors supplemented with acetic acid yielded a rapid depletion of sulfate and nitrate with partial removal of chemical oxygen demand (COD). The COD was reduced from 600 mg/L to a minimum residual COD of 200 mg/L. This is lower than the COD in the original OSPW before acetic acid addition, indicating possible co-metabolic biodegradation of recalcitrant organic compounds.

HiPOx-treated OSPW contained larger amounts of sulfate and a smaller amount of readily biodegradable organic compounds compared to acetic-acid-supplemented OSPW. With longer reaction times, sulfate was depleted and the residual COD was further reduced to 150 mg/L.

The bioreactors that contained MFT-extracted biofilms removed 20% of the naphthenic acids from the acetic-acid-supplemented OSPW and 50% of the COD from the HiPOx-treated OSPW. Further confirmation was obtained from bioreactors using acclimatized biofilms, which removed 30% of the naphthenic acids from the OSPW without HiPOx treatment.

## **Conclusions**

This study demonstrated the feasibility of seeding a biofilm reactor with indigenous microorganisms from MFT. Through monitoring the COD concentration and biodegradation of organic compounds including naphthenic acids with MFT and with biofilms, it was found that the indigenous microorganisms could be introduced to a biofilm reactor to biodegrade recalcitrant organic compounds. The results provide insights on biodegradation of toxic and recalcitrant organic compounds and help the design of a continuous bioreactor for OSPW treatment.

### *Anaerobic Biodegradation of OSPW with MFT and with Biofilms Originated from MFT*

Both MFT and MFT-originated biofilms demonstrated the ability to utilize dissolved organic compounds in OSPW to support different anaerobic microbial processes with the presence of acetic acids. During the batch biodegradation tests, COD was rapidly reduced from 600 mg/L to 200 mg/L in the first 10 days of incubation, indicating that the indigenous microbial community is very active and its activity could be promoted with a supplemented readily biodegradable organic carbon source (in this case acetic acid). Along with the rapid utilization of readily biodegradable substrates, sulfate was depleted in 16 days. Denitrification was observed immediately after nitrate spiking, suggesting that the indigenous microbial community could support multiple anaerobic biodegradation processes.

After biodegradation, the residual COD in acetic acid-supplemented OSPW was reduced to 200 mg/L which is lower than the original COD in OSPW, indicating partial removal of OSPW-originated dissolved organic compounds, potentially by co-metabolism.

### *Anaerobic Biodegradation of HiPOx-treated OSPW with MFT and with Biofilms Originated from MFT*

HiPOx treatment breaks down recalcitrant and long-chain organic compounds into smaller molecules that are less resistant to biodegradation, promoting the biodegradation of dissolved organic compounds. After biodegradation, the residual COD was reduced to 150 mg/L.

Leaching of naphthenic acids from bitumen in the MFT was observed when MFT was present in the bioreactors. Using MFT-originated biofilms (in place of MFT as a source of microorganisms) could achieve the same treatment while eliminating the leaching effects. Consequently, MFT-originated biofilms demonstrated clear advantages in removing dissolved organic compounds in the OSPW.

### *Anaerobic Biodegradation of OSPW with Acclimatized Biofilms from a Bioreactor*

The biodegradation tests with acclimatized biofilms did not display a lag phase at the beginning of the incubation, suggesting that acclimatized biofilms carry a complex microbial community that could sustain the biodegradation processes.

The COD was reduced to 200 mg/L in 20 days, which is at the same level as the best biodegradation tests results with MFT. The results demonstrated that acclimatized biofilms can have the same treatment efficiency as that of MFT, while eliminating the unnecessary interference. In addition, the acclimatized biofilms plus their carriers occupied a much smaller volume (250 mL) than that of MFT (1,000 mL), providing potential to further increase the biomass amount and the treatment efficiency.

Naphthenic acid concentration was reduced from 35 mg/L to 25 mg/L after biodegradation. The decreased naphthenic acid concentration was detected by both HPLC-MS and fluorescence spectrometry, indicating that fluorescence spectrometry<sup>4</sup> could be used to characterize naphthenic acid concentration change in daily bioreactors operations.

Future work should focus on development of bioreactor with continuous influent to encourage continuous growth of biofilms.

Delgado Chavez, L., 2014. Anaerobic degradation of oil sands tailings. University of Alberta, Department of Civil and Environmental Engineering, Edmonton, Alberta. M.Sc. Thesis. 122 pp. <http://hdl.handle.net/10402/era.37318>

#### **Abstract**

The objective of this study was to determine if there was any difference in the bioremediation of oil sands process-affected water (OSPW) between different treatment scenarios and to identify and quantify bacteria present in the different treatments. Two reactors (treatments) were compared in this study: an acetic acid-amended OSPW bioreactor (AAAO bioreactor) and a HiPOx treated OSPW bioreactor (HTO bioreactor). The AAAO bioreactor contained 1,750 mL OSPW and 250 mL of mature fine tailings (MFT). The second bioreactor contained the same amount of OSPW and MFT with the only difference that OSPW was treated with an advanced oxidation process (HiPOx). The AAAO bioreactor was able to remove 70% of COD and 15% of naphthenic acids (NA). The HTO bioreactor removed 48% of COD and 19% of NA under nitrate reducing conditions. Bacterial quantification showed that sulfate reducing bacteria (SRB) were the dominant species at the end of the AAAO bioreactor operation at  $4.2 \times 10^6$  copy number per gram. In contrast, the HTO bioreactor showed that Total Bacteria was the dominant group with  $7.0 \times 10^7$  copy number per gram. A community analysis was performed on both bioreactors. In the AAAO bioreactor bacteria identified were *Acidovorax sp.*, *Acidovorax*

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<sup>4</sup> See the following report for a potential in-situ measurement method: De Corby, R.G., 2013. Development of Silicon-Based Optofluidic Sensors for Oil Sands Environmental Monitoring. OSRIN Report No. TR-41. 19 pp. <http://hdl.handle.net/10402/era.36936>

*ebreus*, *Acidovorax defluvii*, *Cryobacterium psychrotoleans*, *Brachymonas petroleovorans*, and uncultured members of *Desulfocapsa* and *Syntrophacea*. In the HTO bioreactor, identified bacteria were *Acidovorax sp.*, *Hydrogenophaga defluvii*, *Rhodoferax sp.*, *Desulfotomaculum sp.*, *Pseudomonas stutzeri* and uncultured members of *Desulfocapsa*.

## Conclusions

This research showed that the chemical oxygen demand (COD) and sulfate present in fresh oil sands tailings pond water can be biodegraded using an anaerobic bioreactor spiked with bacteria indigenous to MFT. There is evidence of naphthenic acid biodegradation in the reactors.

Treating oil sands with an advanced oxidation process (HiPOx) can mineralize and/or degrade naphthenic acids into simpler compounds. Further biodegradation of the remaining COD can be accomplished by anaerobic reactors, as evidence suggested from the HiPOx treated OSPW bioreactor (HTO bioreactor). It would be useful to test the use of HiPOx process technology coupled with biodegradation to remove of the remaining biodegradable hydrocarbons. Further understanding and optimization of the biodegradation of naphthenic acids is needed. More research is needed to determine if nitrate can help bacteria to biodegrade naphthenic acids and to what extent this biodegradation can take place (i.e., if larger concentrations of naphthenic acid are present). It is to be determined how much nitrate is needed to accelerate naphthenic acid biodegradation in this kind of reactor.

The use of molecular biology allowed us to obtain a complete picture of the microbial processes taking place in the bioreactors. The quantification of sulfate reducing bacteria (SRB) and nitrate reducing bacteria (NRB) populations, along with the identification of species of bacteria in the reactors, support the idea that only a very few select species are present in MFT and are able to synergistically degrade organic matter in this environment.

The identification of specific bacteria that flourished in the acetic acid amended untreated and pre-treated oil sands process-affected water gave specific information as to which organisms are the key players in the biodegradation process. Identifying bacteria before and after adding an external electron acceptor also gave information on which bacteria will dominate the community under each set of conditions.

Further research is needed to envisage the implementation of bioreactors to treat oil sands tailings water as a promising and feasible possibility.

## LIST OF OSRIN REPORTS

OSRIN reports are available on the University of Alberta's Education & Research Archive at <http://hdl.handle.net/10402/era.17209>. The Technical Report (TR) series documents results of OSRIN funded projects. The Staff Reports (SR) series represent work done by OSRIN staff.

### **OSRIN Technical Reports – <http://hdl.handle.net/10402/era.17507>**

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