Anaerobic Treatment of Hexavalent Chromium In Industrial Effluent Using Two-phase Anaerobic Sequencing Batch Reactor

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Abstract

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Hexavalent chromium pollution is a major environmental concern due to its toxicity and extensive use in industry. Industrial effluents containing Cr(VI) have been traditionally treated by chemical reduction, followed by precipitation, or more recently, by removal using non-viable biosorbents. This study investigates the use of a two-phase anaerobic sequencing batch reactor (ASBR) for the treatment of Cr(VI) contaminated wastewater. Simulated wastewater containing potassium chromate and sucrose was introduced into reactor one which contained phase I optimized biomass. Complete sorption and reduction of Cr(VI) to Cr(III) occurred in reactor one, which had a 1:1 volume of treated wastewater to settled cheese whey biomass granules. The chromium-free effluent from reactor one, rich in volatile fatty acids (VFA) was the feed stock or influent for reactor two, where optimum methane production was maintained. Reactor one was operational for about 40 days with a Cr(VI) loading of 25 mg/L-day. Cr(III) derived from the reduction of Cr(VI) occurred in the effluent of reactor one at

levels between 1 to 2.5 mg/L only from about day 28 to the end of the experiment. Selective sequential extraction (SSE) of the spent biomass showed that 45% of the added chromium was in the form of insoluble Cr(III) hydroxides, and the remaining 55% as organo-Cr(III) complexes. VFA levels were eventually reduced in reactor one by about 50%, and soluble COD removal by about 65%. Average methane content in the reactor two biogas was greater than 80%. Based on sorption studies, and using a novel approach whereby sorbed Cr(VI) was interpreted as a "dose" in order to obtain various toxicological indices, models were developed for estimating the amount of Cr(VI)-wastewater that could be treated using plant size unit operations. Leaching tests carried out on spent biomass from reactor one over a five month period demonstrated the non-leacheability of chromium. This study indicated the potential feasibility of using a two-phase ASBR system for the co-treatment of inorganic Cr(VI) contaminated wastewater and high organic loading effluents.

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List of Abbreviations

ASBR	Anaerobic Sequencing Batch Reactor
BOD	Biological Oxygen Demand
C_{Cr}	Concentration of Cr(VI) in the wastewater
C_e	Equilibrium concentration
C_f	Final concentration
CH_4	Methane
C_i	Initial concentration
C_{in}	Incoming concentration
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
Cr	Chromium
Cr(III)	Trivalent chromium
Cr(VI)	Hexavalent chromium
CST	Continuous Stirred Tank
CSTR	Continuous Stirred Tank Reactor
EC50	Concentration that effect 50% of the organisms
EGSB	Expanded Granular Sludge Blanket
FS	Fixed Solids
IC50	Concentration that inhibits 50% of the organisms
ID50	Dose that inhibits 50% of the organisms
k	Rate constant

k_0	Rate constant when $L_{Cr} = 0$
L_{Cr}	Accumulated chromium loading
LC50	Concentration that kills 50% of the organisms
LD50	Dose that kills 50% of the organisms
m	Mass of adsorbent
N	Number of cycles
NOM	Natural Organic Matter
q	Removal of adsorbent
q_{max}	Maximum removal of adsorbent
SBR	Sequencing Batch Reactor
SSE	Selective Sequential Extraction
SMA	Specific Methanogenic Activity
t	Residence time
TCD	Thermal Conductivity Detector
TCLP	Toxicity Characteristic Leaching Procedure
TFS	Total Fixed Solids
TS	Total Solids
TSS	Total Suspended Solids
TVS	Total Volatile Solids
TVSS	Total Volatile Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket
V_B	Volume of settled biomass
V_C	Cycle volume

VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids
x	Mass of contaminant adsorbed

Chapter One

INTRODUCTION

1.1 General Remarks

Heavy metals are considered one of the most hazardous forms of persistent, non-biodegradable environmental pollutants (EPA 1995). Sources of these metals include domestic and industrial effluents, atmospheric deposition, runoff, etc. The heavy metals released from these sources eventually enter and contaminate soil and water by a number of different pathways. Metals can adsorb onto soil components, runoff into surface waters, or leach from soil into underlying groundwater. Inevitably they enter the food chain as well as sources of domestic drinking water. Exposure to heavy metals through ingestion of contaminated food or water poses a grave health risk to humans. Also, there is the potential for serious ecological consequences when these metals enter ecosystems in appreciable amounts.

Over the past years uses of heavy metals such as cadmium, chromium, copper, lead, nickel, and zinc have increased substantially. Chromium contamination of soil, surface water and groundwater, and sediment has become a matter of concern in Canada, and particularly in the province of Quebec, where according to the Quebec Ministry of Environment there were 69 chromium-contaminated sites as of October 2003 (Environment Quebec 2003). Chromium can exist in 9

different oxidation states from (-II) to (VI), but only the (0), (II), (III), and (VI) are common. The most stable of these oxidation states are hexavalent chromium, hereafter referred to as Cr(VI), and trivalent chromium, hereafter referred to as Cr(III). These two oxidation states differ in their properties. Cr(VI) is highly toxic and is a proven carcinogen, mutagen, and redox active metal, while Cr(III) is an essential micronutrient for most organisms, including humans. Many compounds of Cr(VI) are generally soluble in water allowing for great mobility through soil and water compartments, while those of Cr(III) are fairly insoluble and thus less mobile. Some examples of chromium compounds with different oxidation states are listed in the following table:

Table 1-1. Chromium compounds with different oxidation states (Zayed and Terry 2003).

Oxidation State	Example
(+II)	CrF_2, $CrBr_2$, CrSe
(+III)	Cr_2O_3, $CrCl_3$, CrF_3, $CrBr_3$
(+IV)	CrO_2, CrF_4, $CrBr_4$
(+V)	CrO_4^{-3}, CrF_5
(+VI)	$PbCrO_4$, $K_2Cr_2O_7$,

1.2 Objectives of the Research

The objective of this study is to develop a treatment method for the removal of hexavalent chromium from industrial effluents by utilizing a two-phase anaerobic process. A comprehensive study using both short term batch test experiments as well as long term anaerobic sequencing batch reactor (ASBR) studies were used to accomplish these objectives.

The objectives of this study can be categorized as follows:

- To evaluate the potential of viable anaerobic biomass to reduce hexavalent chromium.
- To determine the capacity of the anaerobic biomass to immobilize chromium.
- To investigate the toxicity of hexavalent chromium to the anaerobic process in order to maintain optimum methane production.
- To establish a mechanism for chromium removal.
- To develop a scale-up protocol using formulas developed from both batch and ASBR experiments.

A chart, summarizing the specific batch and ASBR experiments, is presented in Figure 1-1.

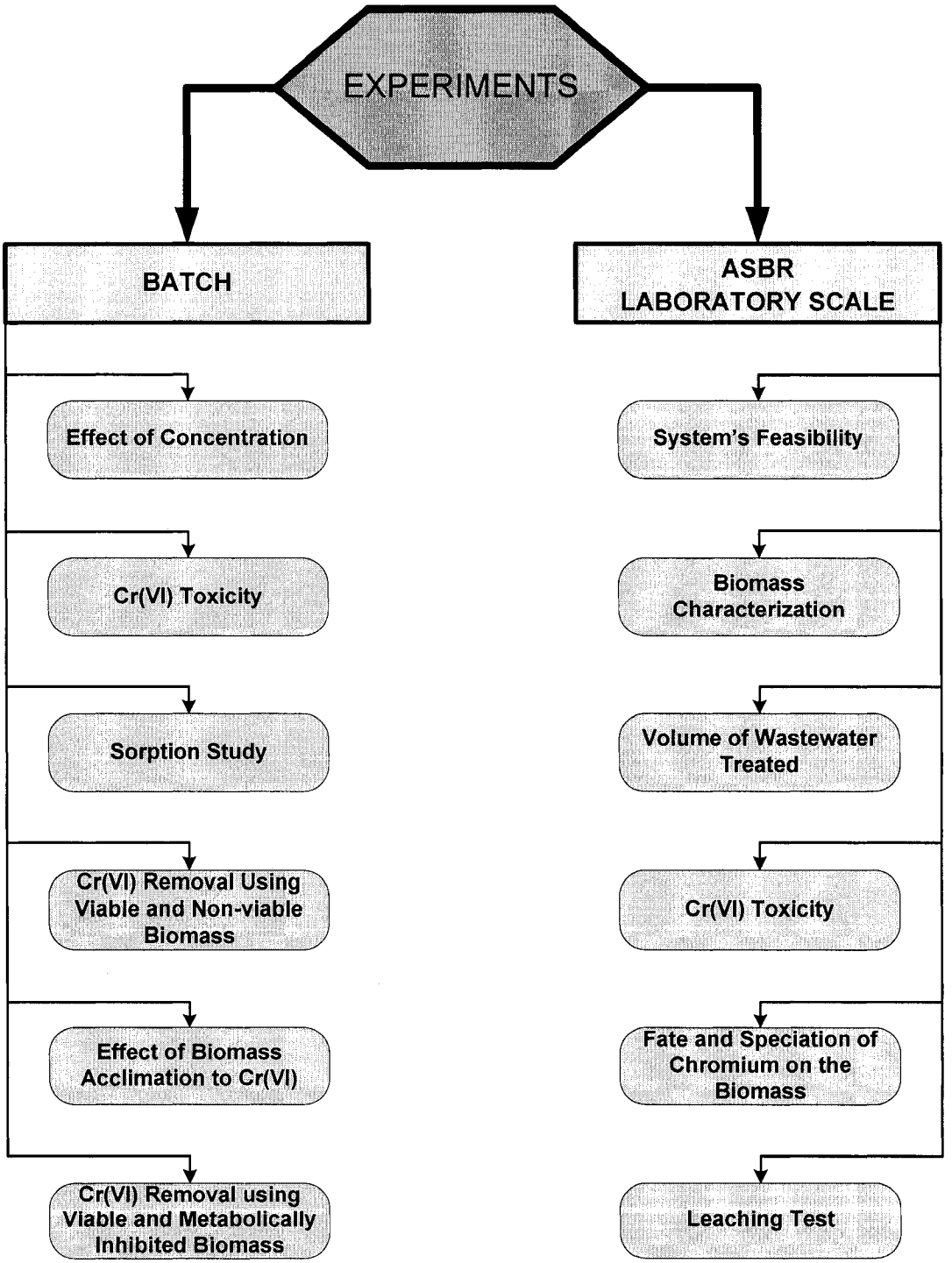

Figure 1-1. Summary of the batch and ASBR experiments

1.3 Organization of the Research Study

This research study is incorporated into eight chapters as follows:

- Chapter One states the problem, the main objectives and the organization of the work.
- Chapter Two provides the literature review on chromium, the anaerobic treatment technology, as well as the concept of biosorption and bioaccumulation of heavy metals. Different studies and findings from previous researchers are summarized and presented.
- Chapter Three includes the description of materials and methods.
- Chapter Four includes results and discussion.
- Chapter Five investigates the fate of hexavalent chromium undergoing biosorption to anaerobic biomass.
- Chapter Six develops models for scale-up based on the toxicity of hexavalent chromium to anaerobic biomass.
- Chapter Seven summarizes the conclusions of this study and states its contributions to knowledge in this area.
- Chapter Eight outlines directions for related future research.

Chapter Two

LITERATURE REVIEW

In this chapter a review of the literature focusing on chromium usage, treatment technologies, and the latest work conducted on chromium remediation, that is involved especially with biologically-mediated reduction, will be presented. A brief background on anaerobic wastewater treatment, its basics, its advantages and disadvantages, as well as literature on biosorption and bioaccumulation are included.

2.1 Chromium

Chromium is the 7th most abundant element in the earth's crust. It is the 24th element in the periodic table (Group VIB) and has an atomic weight of 52 g/mol. Elemental chromium is a grey, lustrous metal that is very resistant to ordinary corrosive agents. *Lehmann* first discovered chromium in 1766 as Siberian red lead (*Plomb rouge de Sibérie*). It was isolated from the natural lead chromate, or crocoite; $PbCrO_4$ (Sully and Brandes 1976).

Canada imports various chromium-containing materials including chromium ferroalloys (55%), chromite ores/concentrates (28%), chromium containing

chemicals (7%), and about 10% miscellaneous chromium-bearing substances such as waste scrap and powder (CEPA 1994).

2.1.1 Applications and Uses

Chromium has many industrial applications which can contribute to chromium pollution through discharges into the environment. Some examples of industries utilizing chromium in their operations are wood treatment, metal plating, stainless steel production, leather tanning, and pigment industries (Barnhart 1997, Katz and Salem 1994).

2.1.2 Chromium in the Environment

2.1.2.1 Chromium in Air

According to the Canadian Environmental Protection Act (CEPA 1994), it is estimated that about 84 tonnes of chromium (both trivalent and hexavalent forms) are released into the Canadian atmosphere from anthropogenic sources each year. The mean airborne concentrations of chromium in 12 Canadian cities ranged from 0.003 to 0.009 $\mu g/m^3$, while levels in non-urban areas were usually below 0.001 $\mu g/m^3$. These concentrations would be higher near cities where several iron and steel mills are located (i.e. in Hamilton, Ontario, a concentration of 0.02 $\mu g/m^3$ was found).

2.1.2.2 Chromium in Water

Chromium enters the Canadian aquatic environment from many industrial sources. Available data showed that 27 tonnes of chromium are released annually in liquid discharges from Canadian base metal smelters and refineries, as well as from iron and steel plants, and metal finishing plants (CEPA 1994).

Average concentrations of total chromium in non-contaminated surface and marine waters are generally below 1.0 μg/L. However, much higher concentrations have been reported in contaminated surface waters ($\leq$ 165 μg/L in British Columbia) (CEPA 1994).

Sediments in many parts of Canada have become contaminated with chromium as a result of loading from industrial sources. As an example, in St. Mary's River system, a concentration of 31,000 mg/kg dry weight of sediment (d.w.) was found and a concentration of 1,920 mg/kg (d.w.) was also found in the Detroit River (CEPA 1994).

2.1.2.3 Chromium in Soil

The presence of chromium in the soil environment is controlled by the three main reactions which are oxidation-reduction, precipitation-dissolution, and sorption-desorption reactions (Zayed and Terry 2003). Soil chromium concentrations from 173 Canadian sites ranged from 10-100 mg/kg (d.w.). However, higher levels were found near chromium industrial facilities. For example, a concentration of

243 mg/kg (d.w.) was reported at a wood preserver's property in Neepawa, Manitoba (CEPA 1994). It should be noted that the Ministry of Environment of Quebec has regulated the concentrations of both total and hexavalent chromium in water and soil as listed in Table 2-1.

Table 2-1. Allowable concentrations of Cr(VI) and total chromium in water and soil in Quebec (Environment Quebec 1999).

Media	Cr(VI) concentration	Total chromium concentration
Drinking Water	0 µg/L	50 µg/L
Surface Water	50 µg/L	200 µg/L
Soil	N/A*	250 mg/kg (residential area) 800 mg/kg (commercial & industrial areas)

* Not available

2.1.3 Treatment Technologies

The existing treatment technologies for hexavalent chromium-contaminated soil, water, and groundwater are based on what is called "remediation by reduction"; which is basically the reduction of hexavalent chromium to the trivalent form. Some examples of in-situ treatment technologies are geochemical fixation, soil flushing, natural attenuation, phytoremediation, permeable reactive barriers (PRBs) and reactive zones. These technologies have been utilized at full-scale and are generally supported by performance and cost data (EPA 2000).

2.1.4 Hexavalent Chromium

Hexavalent chromium can be reduced chemically or biologically under both aerobic and anaerobic conditions. Ferrous iron (Eary and Rai 1988), elemental

iron (James 1994), green rust (Bondand and Fendrof 2003), hydrogen peroxide (Pettine et al. 2002), hydrogen sulfide gas (Thronton and Amonette 1999) as well as many other chemicals such as ferrous sulfate, ferrous ammonium sulfate, sodium sulfite, sodium hydrosulfite, sodium bisulfite, and sulfur dioxide (Higgins et al. 1997) are some well-known compounds that are capable of the chemical reduction of hexavalent chromium. On the other hand, many bacterial cultures (mixed or pure bacteria) are also capable of reducing the hexavalent chromium to the trivalent form. Some examples of these bacteria are: *Shewanella oneidensis* (Lowe et al. 2003) *Streptomyces griseus* (Laxman and More 2002), *Entrobacter cloacae strain HO1* (Lovely 1995), *Bacillus subtilis* (Garbisu et al. 1998), *Pseudomonas mendocina* (Salunkhe et al. 1998), *Streptomyces thermocarboxydus* (Desjardin et al. 2002), *Escherichia coli ATCC 33456* (Wang et al. 2000), *Thiobacillus ferrooxidans* (Quintana et al. 2001), *Shewanella oneidensis MR-1* (Viamajala et al. 2002), iron-reducing bacteria (IRB) (Wielinga et al. 2001), sulphate-reducing bacteria (SRB) (Turick and Apel 1997), rhamnolipids (bacterial product) (Massara et al. 2007) and many more.

Beside the bacterial reduction of hexavalent chromium; different plants such as vascular aquatic plants (Chandra et al. 1997), marine algae (Lee et al. 2000) and wetland plants (Lytle et al. 1998) are capable of Cr(VI) reduction under different levels of chromium accumulation. Soil's natural components have also the ability to reduce Cr(VI) to Cr(III), the natural organic matter (NOM) such as fulvic acid

(Wittbrodt and Palmer 1995) and humic acid (Wittbrodt and Palmer 1996) are some of these components.

2.1.5 Trivalent Chromium

As stated earlier, trivalent chromium is less mobile and toxic than hexavalent chromium, and it is considered essential for most living organisms including humans. Therefore it is not considered a priority list pollutant. However, trivalent chromium pollution problems would arise if it becomes mobilized through oxidation to the more soluble hexavalent form. Solubilization of trivalent chromium can also occur through complexation with naturally occurring ligands, and any subsequent oxidation to the hexavalent species would obviously worsen the situation. It therefore should not be assumed that the presence of Cr(III) in soil or water is harmless (Ross et al. 1981).

Bartlett and James (1979) have found that trivalent chromium can be naturally oxidized within the soil by manganese (hydr)oxides which have a high adsorption capacity for metal ions. The oxidation increased with decreasing pH in old, dried, sieved soil. Another oxidant of aqueous Cr(III) is dissolved oxygen, but the oxidation rate is too slow to be considered a significant factor for Cr(III) oxidation (Eary and Rai 1987).

In another publication, James and Bartlett (1983b) showed that despite the high levels of reducing organic compounds in sludge and soil from tannery wastes,

Cr(III) was oxidized to Cr(VI). Chinthamreddy and Reddy (1999) showed that Cr(III) oxidation depends on the soil pH which, depends on the buffering capacity of the soil. In low-buffering soils, significant oxidation of trivalent chromium can occur. Oxidation can be limited by Cr(III) complexation (or chelating) with organic ligands and compounds. James and Bartlett (1983a) stated that the complexation of Cr(III) with fulvic acid has rendered it mobile in soil and prevented its precipitation.

In a recent study conducted by Massara et al. (2007), trivalent chromium was mobilized (extracted) when biosurfactants (rhamnolipids) were added to soil contaminated with Cr(III). The rhamnolipids had the ability to extract Cr(III) from the carbonate and the oxide/hydroxide fractions of the soil. The optimum parameters were the following: pH 6, the rhamnolipid concentration ranged between 1-1.5%, a contact time of 3 days and a soil to solution ratio of 1g to 20 mL.

2.2 Anaerobic Treatment Biotechnology

2.2.1 Background

Anaerobic treatment of wastewater became popular in the early 1980's (Mulligan 2002). The process employs a group of facultative and strictly anaerobic bacteria to decompose organic material in wastewater. As a result of its successful application for municipal wastewater, it has been applied to the treatment of industrial wastewaters and sludge (Eckenfelder and Santhanam 1981).

Like all other technologies, this process has its own advantages and disadvantages. Some of its advantages are reduction of waste biomass disposal cost, less nitrogen and phosphorous supplementation cost, reduction of installation space requirements, useful end products such as methane gas, elimination of off-gas air pollution, biodegradation of aerobic non-biodegradable pollutants (Speece 1996), low or slow growth rate and a stabilized sludge which signifies that only a small portion of the degradable organic waste is being synthesized into new cells (Tchobanoglous and Burton 1991).

On the other hand, the disadvantages of the process can be summarized as follows: long start up requirement for development of biomass, requirement for heat input to maintain the optimum temperature, the process generation of low effluent quality for surface water discharge in some cases, and lastly, elevated sensitivity to toxic heavy metals and other oxidizing agents which requires dilution in some cases (Eckenfelder and Santhanam 1981, Speece 1996, Lin and Chen 1999).

Many types of anaerobic reactors exist such as the upflow anaerobic sludge blanket reactor (UASB), expanded granular sludge blanket reactor (EGSB), multiplate reactor, anaerobic filter, fixed-film reactor, upflow fixed-film reactor, down flow fixed-film reactor, fluidized bed reactor, anaerobic ponds, anaerobic sequencing batch reactor (ASBR) and two-phase digestion (Mulligan 2002). The

ASBR and two-phase digestion types will receive more attention later because they will form the basis for this research project's treatment protocol.

2.2.2 Concept of Anaerobic Treatment

All anaerobic biological treatment processes involve a consortium of bacteria (from 10^5 to 10^7 bacteria per mL) and are based on a series of reactions (Mulligan 2002). Since methane generation is commonly the rate-limiting step, in most cases primary attention should be given to delineating the most favorable conditions to ensure proper methanogenic activity.

The following reaction is typical of anaerobic digestion:

$$\textbf{Organic matter} + H_2O \rightarrow CH_4 + CO_2 + \textbf{biomass}$$

The microbial consortia carry out a complex biochemical process involving many classes of bacteria and several intermediate steps. Solera et al. (2002) showed that anaerobic digestion can be considered as a three-step process. In the initial stage, complex organic materials are hydrolysed through the extracellular enzymes to basic and simple organic compounds such as monosaccharides and amino acids (Lackey and Hendrickson 1957), then converted to CO_2, H_2 and fatty acids, mainly acetic, propionic and butyric acids by a group of microorganisms that consist of facultative and obligate anaerobic bacteria identified as acidogens or acid formers (Tchobanoglous and Burton 1991). In the next stage, all the higher acids are converted to acetic acid and in the final stage, a biogas

containing methane (CH_4) and carbon dioxide (CO_2) is produced along two different pathways: from acetic acid (acetoclastic methanogens), where the latter is being derived from the carboxyl group and the methane from the methyl group, and from CO_2 and H_2 (H_2-utilizer methanogens). The bacteria in this stage are strict anaerobes, non-spore forming and called methanogenic bacteria (Eckenfelder and O'Connor 1961). A generally accepted controlling factor is that about two-thirds of the methane produced in an anaerobic reactor receiving complex organics is derived from acetate, whereas the remainder is derived from hydrogen and carbon dioxide (Speece 1996). An accepted criterion of the performance of an anaerobic system has been biogas production rather than the removal of organic material (Kountz and Nesbitt 1957). Lastly it should be noted that gas from an optimally performing anaerobic reactor contains 25-35% carbon dioxide and 65-75% methane. A general formula representing the decomposition of organic acids to methane and carbon dioxide is written below (Eckenfelder and O'Connor 1961):

$$\mathbf{C_nH_aO_b + (n - a/4 - b/2)\ H_2O = (n/2 - 9/8 + b/4)\ CO_2 + (n/2 - 2/8 - b/4)\ CH_4}$$

Figure 2-1 provides the metabolic steps in the conversion of organic matter by anaerobic bacteria.

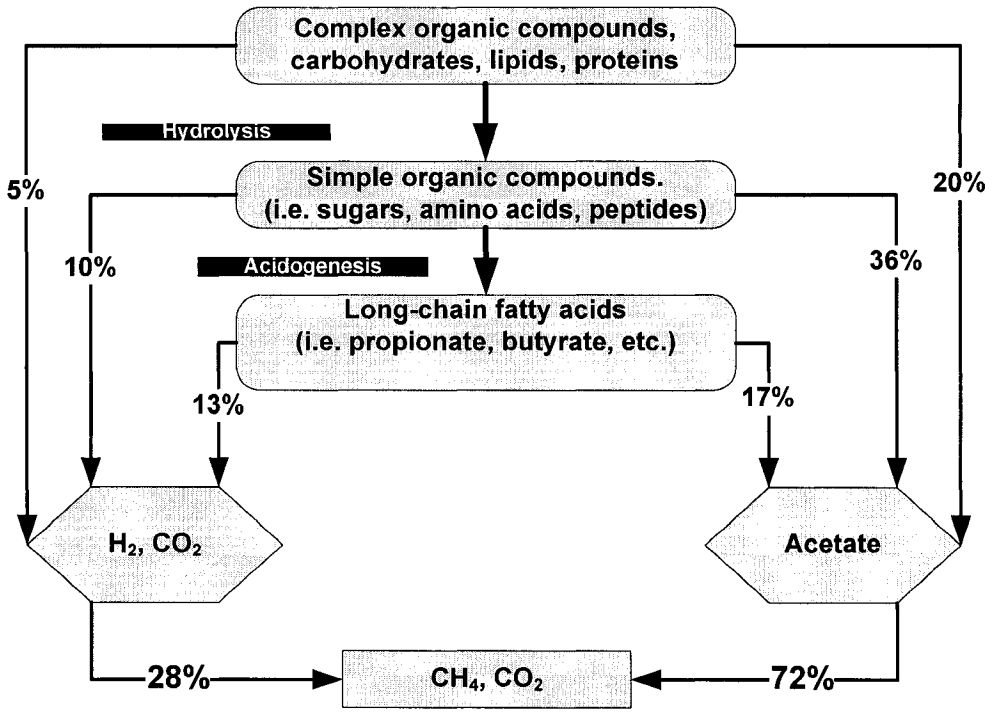

Figure 2-1. Anaerobic decomposition of organic matter resulting in methanogenesis (adapted from Mulligan 2002).

It can be concluded that the anaerobic process works well as long as organic acids are processed as fast as they are produced. However, it should be noted here that an accumulation of the produced organic acids can lead to the digester malfunction or failure if they are not converted to methane and carbon dioxide. Therefore, the conversion of VFAs to CH_4 and CO_2 is considered to be the limiting reaction under dynamic conditions (Speece 1996).

2.2.3 Anaerobic Treatment Requirements

Like any other microbial system, anaerobic digestion is very sensitive to environmental changes inside the system and requires a number of conditions to

maintain its satisfactory functioning (Speece 1996). These conditions can be briefly summarized as follows:

- pH of 6.5 to 8.2 (for methanogenic bacteria).
- Temperature should be in the mesophilic range, 30 to 37° C.
- Adequate amounts of macro- and micronutrients.
- Bioavailable heavy metal trace elements.
- Carbon sources from the organics in the feedstock.
- Electron donors and acceptors that provide energy for the biomass.
- Adequate metabolism time.
- Acclimation to toxic substances in industrial wastewaters.

2.2.4 Two-Phase Digestion

Anaerobic treatment can be operated in single or two phase configurations. Single phase incorporates both acid formation and methane production in the same reactor, while two phase operation attempts to separate the volatile fatty acids (VFAs) formation from methane production, usually by providing two reactors. Some examples of the volatile fatty acids are acetic, propionic, and butyric acid, which play an important role in methane generation. A dramatic improvement in the performance of a CSTR (Continuous Stirred Tank Reactor) has been demonstrated by an acid phase stage prior to the main methanogenic reactor (Speece 1996).

It is very important to maintain a pH range in the acid formation and methane production phases. A pH range of 4 to 6.5 has been reported as optimal for the acid formation step, while a pH range of 6.5 to 8.2 is optimal for methane production, because the activity of methanogenic bacteria can be compromised at pH values below 6.2 (Tchobanoglous and Burton 1991). Because of the optimal pH difference between the two phases, it seems logical to assume that the overall process would operate more efficiently with the phases separated and thus at their optimal environmental conditions.

In this configuration, a selection and enrichment of different bacteria in each digester is possible, the stability of the process increases by controlling the acidification phase hence preventing overloading and build-up of toxic material; the first phase acts as a metabolic buffer which prevents pH shock to the sensitive methanogenic bacteria. In addition, lowering the pH, increasing the organic loading rate and shortening the hydraulic retention time are factors which favor and stimulate the establishment of the acidogenic phase, and prevent the establishment of the methanogenic phase (Solera et al. 2002).

2.2.5 Anaerobic Sequencing Batch Reactor

The sequencing batch reactor (SBR) is a fill-and draw activated sludge system for wastewater treatment where wastewater is added to a batch reactor, treated for the removal of undesirable components, and then discharged (EPA 1999).

Due to its advantages over the continuous process, the anaerobic sequencing batch reactor (ASBR) containing granular biomass has been used for the treatment of wastewater with high organic loading derived from slaughter house, food processing and domestic (sewage) wastes (Massé and Masse 2000). Some of its advantages are better solids retention, efficient operating control, absence of primary or secondary settling, high organic matter removal efficiency, simple operation and no need for biomass separation (Ratusznei et al. 2000, Rodriguez et al. 2004).

As shown in Figure 2-2, the ASBR has five periods of operation in general: fill, react, settle, draw and idle. Biological reactions commence during the fill period. The reactor contents are mixed during the fill and react periods to allow close contact between the bacteria and the solution of organic waste. The mixing should be as gentle as possible to avoid the disruption of granule formation (biomass) (Timur and Ozturk 1999, Massé and Masse 2000).

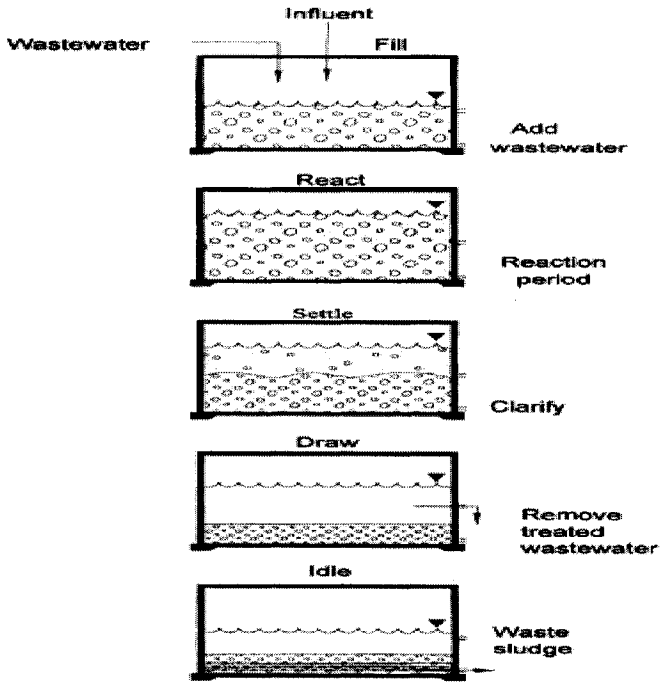

Figure 2-2. Operation of an ASBR (Massé and Masse 2000)

2.3 Biosorption and Bioaccumulation

Biosorption involves the removal of heavy metals from wastewater via adsorption on living or dead biomass in batch, continuous-stirred tanks (CST), fixed-packed beds, and fluidized beds (Mulligan 2002). Gadd (1990) defined it as "the removal of metal or metalloid species, compounds and particulates from solution by biological material".

Volesky (1990) showed that biosorption is a potential alternative to the traditional treatment processes of metal ion removal. It utilizes the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediated or purely physico-chemical pathways of uptake. Biosorption has been

applied to the uptake of metal by biological materials and non-metabolic sorption processes. Biosorption strictly refers to non-active binding, while bioaccumulation more accurately describes metabolism-dependent microbial metal uptake (Gadd 1990, Tobin 2001). As for any other treatment system, biosorption may be influenced by various factors such as pH, temperature, and concentration of both biosorbent and metal ions (Tobin 2001).

The biosorbent consists of one of the following: bacteria, fungus, algae, yeast, plant material, agricultural residues, forestry waste products and many other materials. The use of biomass for the removal of heavy metals depends on the biosorption capacity (mg-contaminant/g-biosorbent), the cost and availability of biosorbent, and ease of regeneration (Mulligan 2002).The source and type of biosorbent is an important factor in heavy metal removal applications. Low cost industrial bio-wastes, naturally available organisms, and quick growing organisms, are some sources of potential biosorbents (Vieira and Volesky 2000).

The advantages of an ideal biosorption process are the following: low cost, reusability of biomass, removal of metals from effluent irrespective of toxicity, short operation times, no production of secondary wastes (such as sludge) which might be toxic, recovery of valuable elements, and ease of operation (Srinath et al. 2002, Aksu et al. 2002).

Both viable (living) and non-viable (dead) biomass can remove metal ions from solution. The removal mechanism is comprised of two phases, an initial rapid phase involving physical adsorption or ion-exchange at the cell surface, and a slower phase involving active metabolism-dependent transport of metal into bacterial cells (for viable biomass). Each one has its own advantages and disadvantages. The use of non-viable biomass does not require continuous nutrient supply, the biomass is not subjected to constraints such as metal toxicity, and the recovery of sorbed metal ions can be performed by an appropriate desorption method (Gadd 1990). On the other hand, the biosorption process using living biomass (bioaccumulation) avoids the need for a separate biomass production process for cultivation, harvesting, processing and storage prior to use (Dursun et al. 2003) and it has more ability to remove metal ions than the non-viable biomass thanks to the wider scope for metal uptake.

Some examples of different types of non-viable biosorbents used for the removal of Cr(VI) anions from solution are *Sphagnum* moss peat (Sharma and Forster 1995), dead fungal biomass (Bai and Abraham 1998), biomass (oat) from *Avena monida* (Gardea-Torresdey et al. 2000), cone biomass from *Pinus sylvestris* (Ucun et al. 2002), dried activated sludge (Aksu et al. 2002), clay and wollastonite (Sharma 2003), distillery sludge (Selvaraj et al. 2003), carrot residues (Nasernejada et al. 2004), *bagasse* fly ash from sugar industry waste (Gupta and Ali 2004), and cationic surfactant-modified yeast (Bingol et al. 2004). Other examples of the bioaccumulation of hexavalent chromium from solutions

involve: green algae of the *Spirogyra* species (Gupta et al. 2001), *Aspergillus niger* (Dursun et al 2003), *Dunaliella* species in saline water (Donmez and Aksu 2002), aquatic *Macrophyte* (Maine et al 2004), as well as biomass from the activated sludge process (Mazierski 1995, Yetis et al. 1999, Stasinakisa et al. 2002, 2003). Table 2-2 provides a brief summary of previously mentioned sorbents and biosorbents, their sorption capacity, and optimal pH conditions.

Inorganic effluents containing heavy metals are not considered candidates for anaerobic treatment due to the low organic content (organic loading) of these effluents. Treatment of these effluents is generally done by chemical and physical means such as chemical reduction, ion-exchange, or precipitation. These treatments are rather costly and responsible for the generation of secondary wastes that are in some cases as hazardous as the original. No other study or investigation, to our knowledge, has been conducted with regards to the treatment by reduction of Cr(VI) in industrial effluents, using the anaerobic treatment process, by exploiting the sorptive capacity of living biomass, as well as its ability to facilitate both chemical and biochemically-mediated reduction of Cr(VI) to Cr(III). The potential exists to mix an inorganic wastewater stream containing Cr(VI) with one containing organic matter, and process the mixture anaerobically using a two-phase ASBR system. The two-phase approach was chosen to sequester the generally more sensitive methane-producing bacterial populations from the toxic effects of hexavalent chromium loading so as to maintain optimum methane production as well as Cr(VI) removal.

Table 2-2. Maximal uptake/removal of chromium using different (bio)sorbents.

Biosorbent	Condition	Initial Cr concentration	q or removal	pH	Reference
Sphagnum	Non-viable	100 mg Cr^6/L	65.9 mg/g	2	Sharma and Forster 1995
Fungal Biomass	Non-viable	50 - 500mg Cr^6/L	27.55 mg/g	2	Bai and Abraham 2003
Avena monida	Non-viable	30 mg Cr^6/L	32%	2	Gardea-Torresdey 2000
Spirogyra	Non-viable	5 mg Cr^6/L	14.7 mg/g	2	Gupta et al. 2001
Dunaliella	Non-viable	100 mg Cr^6/L	45.5-58.3 mg/g	2	Donmez and Aksu 2002
Pinus sylvestris	Non-viable	50 mg Cr^6/L	201.8 mg/g	1	Ucum et al. 2002
Activated sludge	Non-viable	≤ 100 mg Cr^6/dm^3	294.1 mg/g	1	Aksu et al. 2002
Clay and wollastonite	Non-viable	2.6 mg Cr^6/L	69.5%	2.5	Sharma 2003
Distillery sludge	Non-viable	≤ 40 mg Cr^6/L	5.7 mg/g	3	Selvaraj et al. 2003
Surfactant-modified yeast	Non-viable	≤ 208 mg Cr^6/L	99.5%	4.5-5.5	Bingol et al. 2004
Carrot residues	Non-viable	≤ 1350 mg Cr^3/L	45.09 mg/g	4.5	Nasemejada et al. 2004
Bagasse	Non-viable	≤ 10000 mg Cr^3/L	4.35 mg/g	5	Gupta and Ali 2004
Activated sludge	Viable	11 mg Cr^6/L	N/A	N/A	Mazierski 1995
Activated sludge	Viable	25 mg Cr^6/L	N/A	N/A	Yetis et al. 1999
Activated sludge	Viable	25 mg Cr^6/L	N/A	N/A	Stasinakisa et al. 2002
Aspergillus niger	Viable	75 mg Cr^6/m^3	N/A (very toxic)	3.5	Dursun et al. 2003
Aquatic Macrophyte	Viable	≤ 6 mg Cr^6/L	N/A	N/A	Maine et al 2004

Chapter Three

MATERIALS and METHODS

3.1 General Remarks

This chapter describes in detail the materials and methods used in this study. It includes analytical methods for the determination of chromium, chemical oxygen demand (COD), volatile fatty acids (VFAs), and carbon dioxide (CO_2) and methane gas (CH_4) content. In addition, methods used for the characterization of biomass such as total solids (TS), total suspended solids (TSS), total volatile solids (TVS), etc. are presented. Also protocols followed for batch test and ASBR experiments will be outlined. The methodology described in this chapter is directed towards the development of a two-phase anaerobic sequencing batch reactor (ASBR), where the reduction and immobilization of Cr(VI) takes place in the first phase reactor. Figure 3-1 is a simplified schematic of the proposed system.

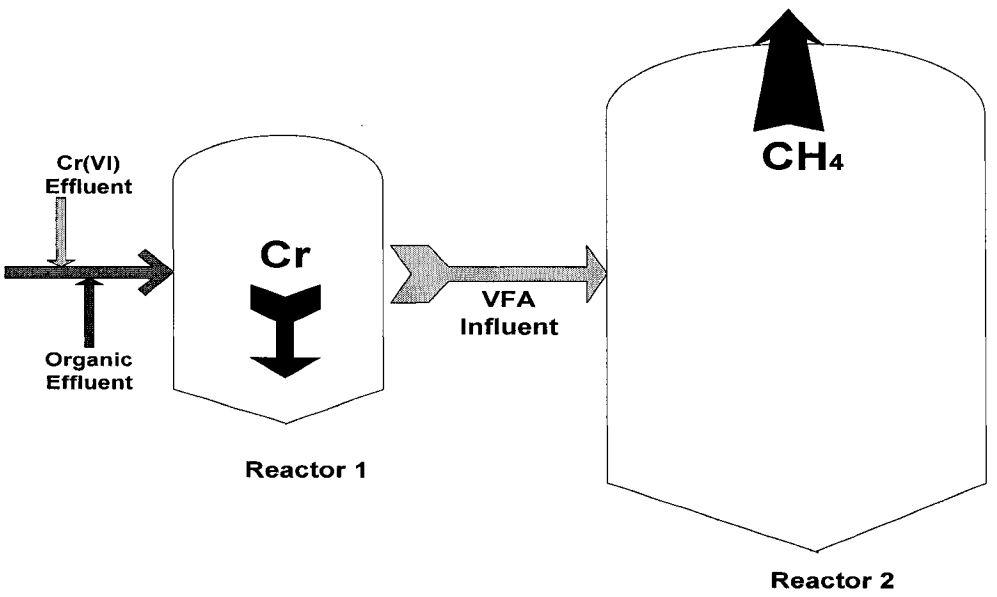

Figure 3-1. Sketch representing the proposed system where Cr(VI) is reduced and immobilized in reactor one.

3.2 Materials

3.2.1 Wastewater

Solutions simulating wastewater used for optimizing phase I and phase II anaerobic activity were prepared from analytical reagent grade chemicals purchased from *Fisher Scientific Canada Ltd.* Table 3-1 shows the composition of the simulated wastewaters (phase I optimizing wastewater was also used for general feeding purposes). These prepared solutions will be referred to as simulated wastewater throughout the body of this work. Solution pHs were adjusted with 5% phosphoric acid according to the application (section 3.2.2).

Table 3-1. Composition of simulated wastewaters (*Armfield Ltd.* 2001)

Component	Phase I optimization wastewater concentration (g/L)	Phase II optimization wastewater concentration (g/L)
Sucrose	8	--
Sodium acetate	--	8
$(NH_4)_2.CO_3$	0.2	0.2
$KH_2.PO_4$	0.4	0.4
$NaHCO_3$	0.4	0.4
1 mL of trace metal solution A		
1 mL of trace metal solution B		
Solution A: $MgSO_4.7H_2O$	5	5
Solution B: $FeCl_3$	5	5
$CaCl_2$	5	5
KCl	5	5
$CoCl_2$	1	1
$NiCl_2$	1	1

3.2.2 Biomass

The anaerobic biomass (granular form) used in this study was obtained from an anaerobic reactor that treats cheese whey generated from a cheese industry *"Agropur"* which is located in Notre Dame du Bon Conseil, Quebec, Canada. The biomass was stored in a refrigerator at 4 °C prior to use.

Before use, the biomass was stored in an incubator at 36±1 °C, for one month in plastic tanks, for regeneration or activation purposes. Biomass was maintained by feeding it with a simulated wastewater, with a pH of about 7.0, and containing sucrose as substrate. The simulated wastewater supernatant was replaced every four days. A "phase I optimized biomass" was prepared by maintaining biomass as above, except that the pH was adjusted to 5.5 and the supernatant replaced

every day to promote phase I anaerobic activity. A "phase II optimized biomass" was also prepared by feeding biomass with simulated wastewater which had a pH of 7 and contained acetate as substrate (see Table 3-1). After a one month optimization period, the CO_2 and CH_4 content of the biogas was measured by placing 12 mL of each settled biomass in serum bottles with 10 mL of the appropriate feedstock. The vials were sealed and sparged (see section 3.4.3) with nitrogen gas and placed in an incubator set at 35°C for 24 hours whereupon the CO_2 and CH_4 content of the headspace was determined by gas chromatography.

3.2.3 Chemicals

All chemicals used were of analytical reagent grade and purchased from *Fisher Scientific Canada Ltd.* The distilled water was prepared using a *Barnstead* Model A1011 distillation unit.

3.2.4 Instrumentation

The following instruments were used in this study:

- Gas Chromatograph (GC), Varian Model "*CP-3800*", for the measurement of CO_2 and CH_4 gas.
- UV/VIS Spectrophotometer, "*Perkin Elmer* Model *Lambda 40*" for the measurement of Cr, COD and VFAs.

- Fermentation unit, "*New Brunswick Scientific* Model *Bioflo 110 Fermentor/Bioreactor*", equipped with a temperature controller, pH adjustment, dissolved oxygen (DO) probe, condenser, and agitator.
- Fisher Scientific Isotemp Incubator Model 304.
- New Brunswick Scientific Innova 2000 platform orbital shaker.
- Denver Instrument M-220 analytical balance.
- Mettler Toledo PB 1502-S top loading balance.
- Fisher Scientific Isotemp Multifurnace.
- Lindbreg/Blue M gravity oven.
- Oakton PH 10 series pH meter (with ORP probe).

3.2.5 Other Materials

Sealable serum glass bottles, COD test kit, syringe filters (*Millex,* pore size 0.45 µm), filter papers (*Whatman* #1), syringes, needles, self-sealing rubber stoppers, aluminum retaining collars for rubber stoppers, and centrifuge tubes were purchased from *Fisher Scientific Canada Ltd.* Glassware was washed with soap and hot water, rinsed multiple times with tap water, rinsed two times with distilled water, and then air-dried prior to use.

3.3 Methods

In this section, a brief description of the methods used for determining chromium (Cr), chemical oxygen demand (COD), volatile fatty acids (VFAs), carbon dioxide (CO_2) and methane gas (CH_4), and biomass characterization is provided.

3.3.1 Total Chromium and Hexavalent Chromium Determination

Hexavalent chromium, in the low range (0-1 mg/L), was determined colorimetrically by reaction with diphenylcarbazide in an acidic solution to produce a red-violet colored complex which absorbs light at 540 nm (adapted from Method 3500-Cr B APHA et al. 1998).

The following steps show the procedure for measuring Cr(VI) colorimetrically by UV/VIS spectrophotometer at a wavelength of 540 nm:

a. 0.1 g of chromium reagent (diphenylcarbazide) was added to 10 mL of sample containing Cr(VI) to develop the red-violet color complex.
b. The mixture was shaken for one minute and left for 3 minutes to react and settle.
c. The sample was then filtered using *Whatman #1* filter paper.
d. The sample was then transferred to 1 cm quartz cells and the concentration of Cr(VI) was measured with reference to a standard curve for known Cr(VI) concentrations.

To determine total chromium, the sample was acidified with sulfuric acid (pH 2) and then oxidized with potassium permanganate before reacting with the diphenylcarbazide. This procedure oxidizes any Cr(III) to Cr(VI). Since any original hexavalent chromium cannot be oxidized (since it is already in the oxidized state), analyzing for Cr(VI) allows for the determination of total

chromium. Trivalent chromium is calculated indirectly from the difference between total and hexavalent chromium.

The following steps summarize the oxidation procedure:

e. 50 mL of the sample were poured into a 125 mL conical flask.
f. 5 mL of H_2SO_4 (5N) were added to the sample (drop wise).
g. The flask contents were brought to a gentle boil on a hot plate.
h. Two drops of $KMnO_4$ (0.5%) were added to give a dark red color.
i. The addition of $KMnO_4$ was continued until the color stabilizes for a certain period of time (approximately 10 min).
j. 5% sodium azide (NaN_3) was added drop wise to the sample until the red color fades completely and a transparent solution was obtained.
k. The sample was then cooled down to room temperature.
l. The volume was adjusted to the original value (50 mL).
m. Then the previous steps (a-d) were repeated as per Cr(VI) determination.

A reference curve was prepared for hexavalent chromium at the following concentrations using a 50 mg/L Cr(VI) stock solution: 0, 0.2, 0.4, 0.6, 0.8 and 1 mg/L.

3.3.2 Chemical Oxygen Demand Measurement

The following colorimetric method (closed reflux method 5220 D. APHA et al. 1998) was used for COD determinations:

I. The COD block heater (*Bioscience Inc.*) was set at 150°C.

II. 2.5 mL of sample (filtered using a 0.45 µm syringe filter) was slowly added to each vial such that it formed a layer on top of the reagent.

III. Each tube was capped and mixed thoroughly by shaking.

IV. Samples were then placed in the COD block heater for 2 hours.

V. Vials were removed from the block heater, left to cool to ambient temperature and wiped clean.

VI. Absorbance at 600 nm was measured for each vial spectrophotometrically.

A reference curve was prepared from potassium hydrogen phthalate (KHP) at the following concentrations: 0, 100, 200, 300, 400 and 500 mg/L. The stock solution of KHP was prepared by dissolving 212.5 mg of KHP in 500 mL distilled water, giving a theoretical chemical oxygen demand of 500 mg/L, as specified by the method.

3.3.3 Volatile Fatty Acids Measurement

This method measures the concentration of volatile fatty acids colorimetrically in the range of 27 to 2800 mg/L at a wave length of 495 nm. It is based on esterification of the carboxylic acids present in the sample and subsequent

determination of the esters by the ferric hydroxamate reaction. The volatile acids in the sample are reported as their equivalent mg/L as acetic acid (Hach Company 2003).

The following steps were used for the measurement of the volatile fatty acids:

1. 1.5 mL of ethylene glycol were added to 0.2 mL of a filtered sample (filtered using 0.45 μm syringe filter), then swirled to mix in a polypropylene tube.
2. 0.2 mL of sulfuric acid (19.2 N) standard solution were then added to the mixture in the tube.
3. The sample was then placed in a beaker with boiling water for a period of three minutes.
4. 0.5 mL of hydroxylamine hydrochloride solution were added to the mixture after cooling it with running tap water.
5. A 2 mL solution of sodium hydroxide (4.5 N) was then added and mixed by swirling.
6. A 10 mL solution of ferric chloride/sulfuric acid was added to the previous mixture.
7. The mixture was then diluted by adding 10 mL of distilled water and left for a three minute reaction period.

The reference curve was prepared using the following concentrations, 0, 350, 700, 1400, and 2800 mg/L of acetic acid, after an appropriate dilution of a supplied standard solution (62,500 mg/L).

3.3.4 Carbon Dioxide and Methane Measurement

This method was adapted from the EPA (1994). A gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) was used to separate and quantitate both carbon dioxide and methane gases in the biogas sample. The sample was introduced into a *Varian* type 1041 On-Column Injector, fitted with a *Valco Instruments Co. Inc.*(VICI) pressurized valve delivery system, as a 0.2 mL sample plug. The TCD detector consists of a thermal conductivity cell in which a Wheatstone bridge is used to compare the temperature of two electrically heated wires, one in the sample gas and the other in the reference carrier gas. Differences in thermal conductivity between sample and reference flows result in different thermal conductivities which are registered as millivolt spikes or peaks (Grune et al. 1957).

The first peak to emerge was that for CH_4, which had an average retention time of about 7.5 minutes. The second peak to emerge was CO_2 which had an average retention time of about 13 minutes. Sample gas peak heights for CO_2 and CH_4 were compared to peaks for standard purified carbon dioxide (99.9%) and methane gases (99.97%), obtained from Praxair. The peak height for the standard CO_2 gas was 4.34 mvolts, and that for the standard CH_4 was 3.52

mvolts. Method conditions are given in Table 3-2. Figure 3-1 shows a typical chromatogram with peaks for methane and carbon dioxide. This chromatogram was obtained from an injection of a biogas sample taken from reactor two during the ASBR experiment (section 4.2).

Table 3-2. Conditions used for GC determinations

Parameter	Specification
Carrier gas	Helium
Detector	TCD
Detector temperature	220 °C
Sample delivery	VICI pressurized valve system
Gas sample volume	0.2 mL
Column flow	5 mL/min
Column oven program temperature	50-100 °C, 5 °C per minute for 15 minutes
Column type	SUPELCO-Carboxen 1010 Plot
Column dimensions	30 m x 0.53 mm
Injector	1041 On-column
Injector temperature	225 °C

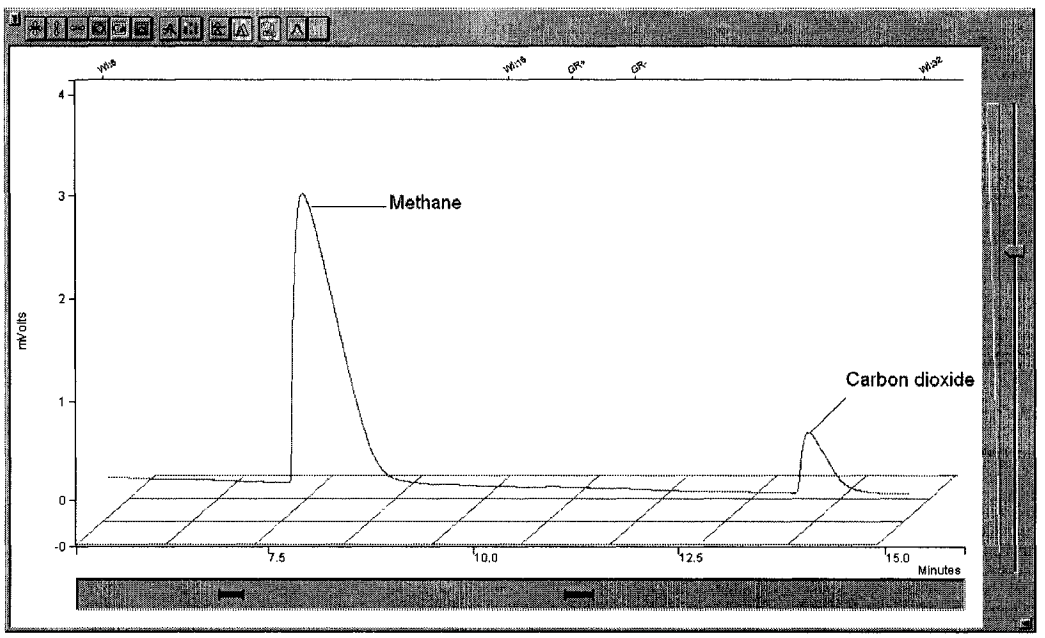

Figure 3-1. A typical chromatogram with peaks for methane and carbon dioxide

3.3.5 Biomass Solids Characterization

Biomass solids were characterized according to American Public Health Association (APHA et al. 1998) standard methods. The original cheese whey settled biomass volume after a 30 day acclimation period for each was determined using Method 2540 F. An Imhoff cone was filled to the 1 liter mark with a well mixed sample of original cheese whey anaerobic biomass. The sample was left to settle for one hour. The volume of the settled biomass was then determined. The settled biomass volume for reactors one and two, after the completion of the ASBR experiment was determined by removing one liter of the liquid phase, after a short settling period (5 minutes), and pouring the remaining biomass into an Imhoff cone and proceeding as above.

Total solids (TS) content of the settled biomass was determined by filling 12 mL, pre-weighed porcelain crucibles with settled biomass. The crucibles were dried to constant weight in an oven at 105 °C. The increase in weight over the empty crucible represents the total solids. Total volatile solids (TVS) for the settled biomass were determined by Method 2540 E by igniting the residue from the previous TS determination in a furnace at 550 °C. The weight loss on ignition represents the volatile solids. Total fixed solids (TFS) for the settled biomass were calculated from the difference between TS and TVS. Both TS and TVS determinations were performed in triplicate.

Total suspended solids (TSS) content of the liquid phase were determined by Method 2540 D by filtering 10 mL aliquots of the liquid phase, from the Imhoff cone settling experiment, through pre-weighed gooch crucibles fitted with standard glass microfiber filters. The crucibles were dried to constant weight at 105 °C. The weight increase represents TSS. Total volatile suspended solids (TVSS) were determined by Method 2540 E by subsequently igniting the crucibles at 550 °C. The weight loss represents TVSS. Total fixed suspended solids were calculated as the difference between TSS and TVSS. Both TSS and TVSS determinations were performed in triplicate.

3.4 Experimental Design

In this section, a detailed procedure for the batch tests and the ASBR experiments is given.

3.4.1 Calculation of the Bulk Water in Settled Biomass

The amount of water in the settled biomass is an important consideration with respect to final estimated chromium levels in subsequent test samples. Ten 12 mL samples of settled biomass (5 minutes settling period) were centrifuged at 3000 rpm for 10 minutes. The supernatant was poured into 10 mL graduated cylinders to estimate the volume of bulk water.

3.4.2 Screening Test for Trivalent and Hexavalent Chromium Solubility

Volumes of 0.4 mL of a 25000 mg/L Cr(III) and Cr(VI) solution prepared from $Cr(NO_3)_3.9H_2O$ (chromium nitrate) and K_2CrO_4 (potassium chromate) were diluted to 20 mL using distilled water and a sodium hydroxide (0.1 N) solution, to yield final concentrations of 500 mg/L each at a pH of 5.5. Samples were prepared in triplicate and shaken for 24 hours at 35 °C. After centrifugation the supernatants were filtered using *Whatman* #40 filter paper and chromium was determined in the filtrate.

3.4.3 Batch Experiments

All batch experiments were carried out on non-optimized, phase I optimized, and phase II optimized biomass. Non-optimized biomass was maintained at pH 7.0 with sucrose as substrate, phase I optimized biomass was maintained at pH 5.7 with sucrose as substrate and phase II optimized biomass at pH 7.0 with acetate as substrate. Batch tests were performed using 50 mL glass serum bottles sealed with rubber stoppers and aluminum collars to ensure biogas retention. All concentration and time studies were carried out for each biomass.

Each serum bottle contained: 8 mL of simulated wastewater, 12 mL of settled biomass, 2 mL of Cr(VI), and 1 mL volume for pH adjustment, bringing the final working volume to 23 mL. Each sealed bottle was sparged with nitrogen gas for three minutes. Nitrogen gas was introduced by a syringe needle into the bottle through the self-sealing rubber stopper or septum to displace air to facilitate

anaerobic conditions as quickly as possible. Air displacement was achieved by simultaneously venting the bottle head space with another syringe needle used as an outlet. After two days, 10 mL headspace samples were collected using a gas-tight syringe and analyzed for CO_2 and CH_4 contents by gas chromatography. Subsequent to head space sampling, each bottle was opened and the soluble COD of the supernatant was measured after filtration using a 0.45 μm syringe filter. The chromium content (hexavalent or trivalent chromium) in the supernatant was also measured. The effect of Cr(VI) on biogas content and COD removal was examined.

To determine the relative degree of phase optimization 12 mL of each biomass were placed in serum bottles with 8 mL of their respective substrate solutions and 3 mL of distilled water. The relative CO_2 and CH_4 contents of the biogas were determined after 24 h at 35 ^{o}C.

3.4.3.1 Concentration Study

A stock solution of 25,000 mg Cr(VI)/L was prepared by dissolving 23.3 g of analytical grade potassium chromate (K_2CrO_4) supplied by *Fisher Scientific* in 250 mL of distilled water. Initial addition solutions of 390.6, 781.3, 1562.5, and 3125 mg Cr(VI)/L were prepared from the stock solution. The concentration study was carried out in serum bottles as per the batch experiment described in section 3.4.2. Two milliliters of the above chromium solution were added to each serum bottle. All final test concentrations were seven-fold dilutions of the above initial

addition concentrations. The effects on COD reduction and biogas content were determined as previously mentioned. This protocol was carried out on the optimized phase I, optimized phase II, as well as non-optimized biomasses. Since anaerobic digestion is a temperature-sensitive process, all experiments were carried out in an incubator set to within the mesophilic operational range (35±1 °C).

3.4.3.2 Sorption Study

3.4.3.2.1 Biomass Preparation

Viable biomass was processed for the equilibrium and sorption isotherm studies by passing phase I optimized biomass through a no. 10 sieve (pore diameter of 2 mm) to eliminate any damaged granules. Retained granules were weighed and used in the sorption study. Non-viable biomass was prepared by drying phase I optimized biomass at 50 °C for 5 days. The dried granules were crushed with a mortar and pestle and passed though no. 30 and 50 sieves in series (having an effective pore diameter of 0.595 and 0.297 mm). Material that was retained on a no. 50 sieve was weighed and used in the sorption study.

3.4.3.2.2 Equilibrium Study

Equilibrium experiments are required before adsorption isotherm studies to determine when equilibrium is achieved between the sorbent (biomass) and the contaminant (hexavalent chromium). Eight samples of 0.5 g (wet weight) viable biomass were placed in centrifuge tubes to which 20 mL of 200 mg Cr(VI)/L,

adjusted to pH 5.5, was added. The same procedure was followed for non-viable biomass except that 20 mL of 100 mg Cr(VI)/L was added to 1 g (dry weight) of biomass. The Cr(VI) solutions were placed overnight in an incubator set to 35 °C prior to addition to the biomass. Sample tubes were also incubated at 35 °C prior to analyses. The residual concentration of Cr(VI) was measured in the supernatant by taking one tube every 15 minutes. After centrifugation and filtration Cr(VI) and Cr(III) levels in the supernatant were determined. Control tubes containing 20 mL of 200 mg Cr(VI)/L without biomass were used to determine if Cr(VI) was lost from solution (or reduced) through sorption to the walls of the polypropylene tubes. In addition, the centrifuged biomass pellets were digested and analyzed for total chromium.

3.4.3.2.3 Sorption Isotherm Study

The sorption isotherm experiment was also carried out in triplicate using 2 g of viable biomass and 20 mL each of a 0 (distilled water), 50, 125, 250, 500, 1000, 1500 and 2500 mg Cr(VI)/L. Biomass and Cr(VI) solutions were placed in 50 mL polypropylene centrifuge tubes and placed on an orbital shaker set to 70 rpm for 24 hours in an incubator set to 35°C. In addition, a triplicate control using 20 mL of 500 mg Cr(VI)/L without biomass was prepared. This control was used to determine if Cr(VI) was being sorbed to the tube walls. The same procedure is carried out in triplicate using 0.5 g of heat-dried non-viable biomass and 20 mL each of a 0 (distilled water), 10, 50, 100, 125, 250, 500, and 1000 mg Cr(VI)/L. The Cr(VI) solutions were prepared from serial dilutions

of a 2500 mg/L stock solution and adjusted to pH 5.5. All the above stated Cr(VI) concentrations were the calculated values based on weight and volume solution preparation measurements; but all initial concentrations in the sorption isotherm experiment were measured values.

The adsorption isotherm was obtained by plotting the amount of contaminant (Cr(VI) in this case) adsorbed per unit mass of adsorbent (biomass) against the concentration of contaminant in the bulk fluid after equilibrium. There are different mathematical forms for adsorption isotherms: linear, Freundlich and Langmuir to name a few of the most generally used (Reynolds and Richards 1995). All of these isotherm models were screened for best fit of the sorption data obtained in this experiment.

The linear model was represented by the following equation:

$$\frac{x}{m} = K_P.C_e \qquad \textbf{3-1}$$

where x = mass of contaminant adsorbed (mg)

m = mass of adsorbent (mg)

C_e = equilibrium concentration of contaminant in the bulk fluid (mg/L)

K_p is an empirical constant which can be determined from the laboratory studies by plotting of x/m vs. C_e

On the other hand, the Freundlich isotherm is an empirical model, expressed mathematically as follows:

$$\frac{x}{m} = K.C_e^{1/n} \qquad \textbf{3-2}$$

where x = mass of contaminant adsorbed (mg)

m = mass of adsorbent (mg)

C_e = equilibrium concentration of contaminant in the bulk fluid (mg/L)

K, *n* are empirical constants which can be determined from the laboratory studies by logarithmic plot of x/m vs. C_e

The third adsorption isotherm model is the Langmuir model, where a single contaminant is involved. This isotherm was developed by assuming that a fixed number of adsorption sites are available, that the adsorption is reversible, and that the adsorbed solute on the adsorbent surface is only one molecule in thickness.

The Langmuir equation is presented as follows:

$$\frac{x}{m} = \frac{q_{\max}.K.C_e}{(1 + K.C_e)} \qquad \textbf{3-3}$$

where x = mass of contaminant adsorbed (mg)

m = mass of adsorbent (g)

C_e = equilibrium concentration of contaminant in the bulk fluid (mg/L)

q_{max}, is the mass of adsorbed solute required to saturate completely a unit mass of adsorbent (mg/g)

K, is an experimental constant

3.4.3.3 Biomass Acclimation to Hexavalent Chromium

Since bacterial cultures can develop resistance to chromate toxicity when grown on chromate amended media (Srinath et al. 2001), phase I optimized biomass was acclimated over a two month period to Cr(VI). One liter of simulated wastewater (containing sucrose and adjusted to pH 5.5) containing 1 mg Cr(VI)/L was added every two days to 500 mL of settled cheese whey biomass, after decanting the previous feedstock supernatant. A control biomass stock was similarly fed with an identical simulated wastewater but without Cr(VI). After the two month period, settled biomass was used in batch tests to assess any difference between chromate-acclimated and control biomass with respect to biogas composition and COD removal. Three concentrations of 55.6, 111.6 and 446.4 mg/L of Cr(VI) were tested against acclimated and control biomass to assess the effect of acclimation following the same protocol used in the concentration study (section 3.4.3).

3.4.3.4 Hexavalent Chromium Removal by Viable and Metabolically Inhibited Biomass

Varying amounts of a 5 % sodium azide (a metabolic inhibitor) solution, were added to a specific volume of active biomass. Five samples of 12 mL each of biomass, one control and four poisoned by 0.25, 0.50, 1 and 2 mL of 5% sodium azide were placed in fermentation tubes, fed with 10 mL of simulated wastewater containing sucrose (pH 5.5), and were placed in the incubator for 24 h at 35°C. Gas production was evident in the control tube, whereas, all additions of sodium azide exhibited no gas production. Figure 3-2 is an illustration of the fermentation tube technique used to determine the viability based on biogas production.

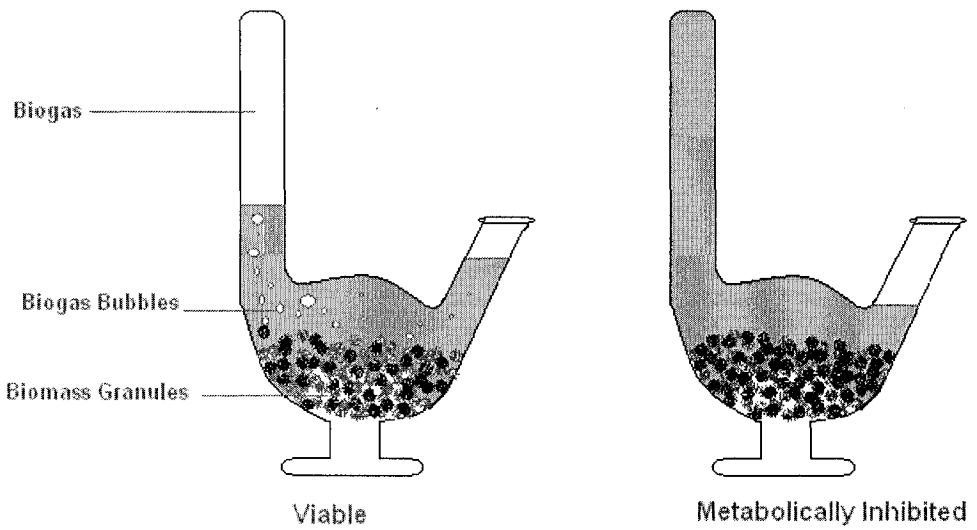

Figure 3-2. Illustration of the use of fermentation tubes.
Produced biogas is trapped in the sealed side arm chimney by displacing liquid.

A comparison of chromium removal was made between viable and metabolically inhibited biomass (0.25 mL sodium azide per 12 mL biomass). A triplicate

analysis using one gram of both types of biomass plus 20 mL of 100 mg Cr(VI)/L was prepared. The tubes were placed in the incubator at 35°C for a period of one day. Subsequently a 1 mL aliquot from the supernatant of each test sample was taken and analyzed for chromium.

3.4.4 Laboratory Scale ASBR Experiment

A laboratory or bench top scale experiment was conducted using a two-phase ASBR protocol with two in tandem reactors: reactor one and two. The primary rationale was to isolate methanogenic activity as much as possible in a chromium-free environment in reactor two. Chromium carry over to reactor two can be controlled using a two-phase reactor design. The secondary rationale was to maintain optimum conditions for phase I anaerobic biodegradation (VFA production) in reactor one, and phase II anaerobic biodegradation (methanogenesis) in reactor two. The feedstock for reactor one was a solution of Cr(VI) mixed with a simulated wastewater containing a soluble, readily biodegradable substrate. The concentration of Cr(VI) was up to two times higher than levels found in inorganic waste streams to ensure the treatment's robustness. In each reactor pH was controlled so as to promote phase I and phase II anaerobic biodegradation respectively. Once chromium removal and substrate conversion to soluble VFAs has been achieved in reactor one, the liquid phase was fed to the biomass in reactor two where conversion of VFAs to methane was achieved. Thus, in addition to pH control, substrate type was

utilized to promote phase I and II anaerobic biodegradation in reactors one and two.

The anaerobic sequential batch experiment was performed using a *Bioflow Fermentor/Bioreactor* fermentation unit. The unit had two reactors with a working volume of two liters each. As stated earlier, each reactor was equipped with a heat jacket for temperature control, a dissolved oxygen probe, to monitor the desired anaerobic conditions, a pH probe, a pump for pH adjustment, an agitator, and a condenser to reduce evaporation of water from the reactors. Figure 3-3(a) is a photograph of the fermentation unit set up. A schematic of the fermentation unit is illustrated in Figure 3-3(b).

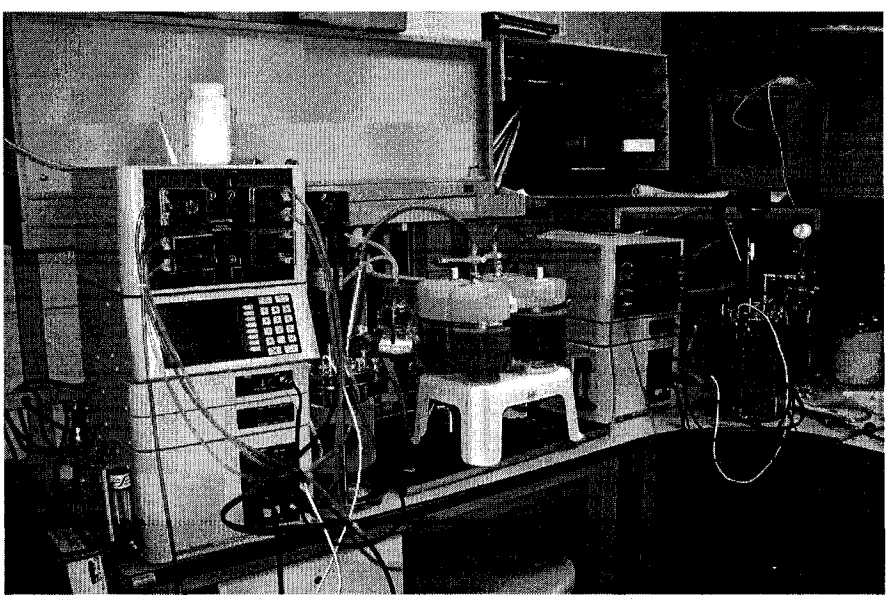

Figure 3-3(a). Photograph of the fermentation unit.

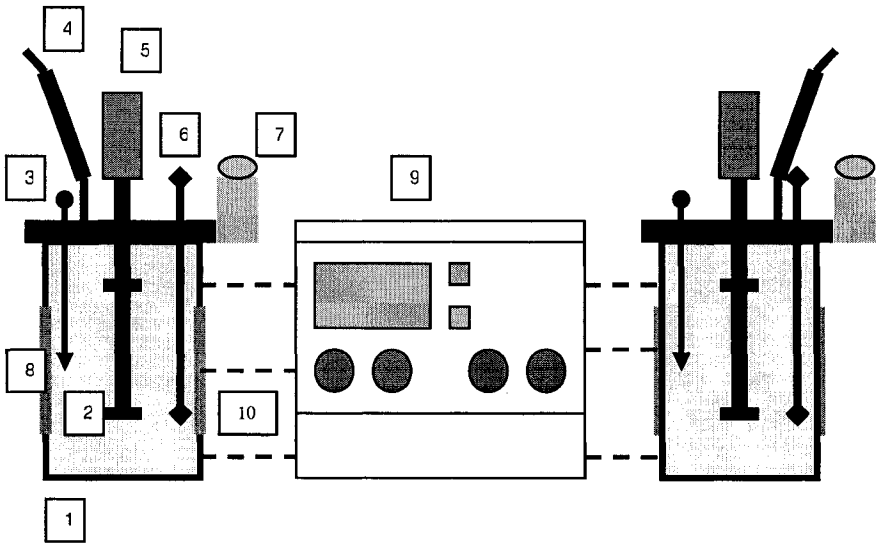

Figure 3-3(b). Schematic of the fermentation unit

1- Reactor	**5- Motor**	**9- Control Unit**
2- Agitator	**6- DO probe**	**10- Tubes**
3- pH probe	**7- pH Solution**	
4- Condenser	**8- Heat Jacket**	

Initially one liter of non-acclimated, well mixed biomass was placed in each reactor. The feedstock for the first reactor was simulated wastewater (pH 5.4) containing sucrose. The second reactor was fed with simulated wastewater (pH 7) containing acetate (Table 3-1). Initial biomass characteristics such as total solids (TS), total volatile solids (TVS), total suspended solids (TSS), total volatile suspended solids (TVSS), fixed solids (FS), and settleable solids were measured.

The temperature of the two reactors was set to 35 ^{o}C using the temperature control option of the fermentation units. An alkaline solution (4% NaOH) was automatically pumped to the first reactor using the system's feedback pH control

option to offset pH decreases due to VFA production. The pH was maintained at a value of 5.4 ±0.2 for the first reactor. The pH control pump used an acidic solution (3.5% phosphoric acid) to maintain the pH at 7.0 ±0.2 throughout the experiment for the second reactor. Without adjustment, the pH in the second reactor tended to increase. Any dilution due to pH adjustment was taken into account when determining COD and VFAs concentrations. Volumes of added pH control solutions were tabulated throughout the experiment.

A phase optimization period for reactor one was established by changing the feedstock (sucrose, pH 5.4) daily. Reducing retention time minimizes the conversion of formed VFAs to methane. A phase optimization period for reactor two was established by changing the feedstock (acetate, pH 7.0) every three days in order to have a maximum conversion of VFAs to methane. This optimization period was extended for thirty days. The initial and final COD and VFA concentrations were determined for each reactor. Although the working volume of both reactors was the same (one liter liquid phase: one liter settled biomass) the hydraulic detention time for reactor one was one day and that for reactor two was three days. Thus in order to maintain proper flow and contact times through the system the experimental protocol required staggering in the following manner:

- simulated wastewater containing sucrose and Cr(VI) was added to reactor one (target temperature for reactor one was reached fairly rapidly after the addition of simulated wastewater)

- after 24 h the supernatant from reactor one was added to reactor two after pH adjustment
- the following two 1-day retention time supernatants removed from reactor one were placed in a fridge at 4 °C after pH adjustment
- after a retention time of three days the supernatant from reactor two was removed and the stored reactor one supernatants were mixed and became the influent for reactor two
- while withdrawing supernatant from reactor two, which required 1.5 h, the mixed supernatants from reactor one were placed in an incubator set to 35 °C prior to addition to reactor two (the temperature readout/control system of the fermentation unit showed that the target temperature for reactor two was reached fairly rapidly)
- this process was continued for the duration of the experiment
- initial influent and final effluent COD and VFA levels were measured for both reactors and initial and final chromium levels were determined for reactor one.

Figure 3-4 is a schematic of the addition sequence protocol for reactors one and two as outlined above.

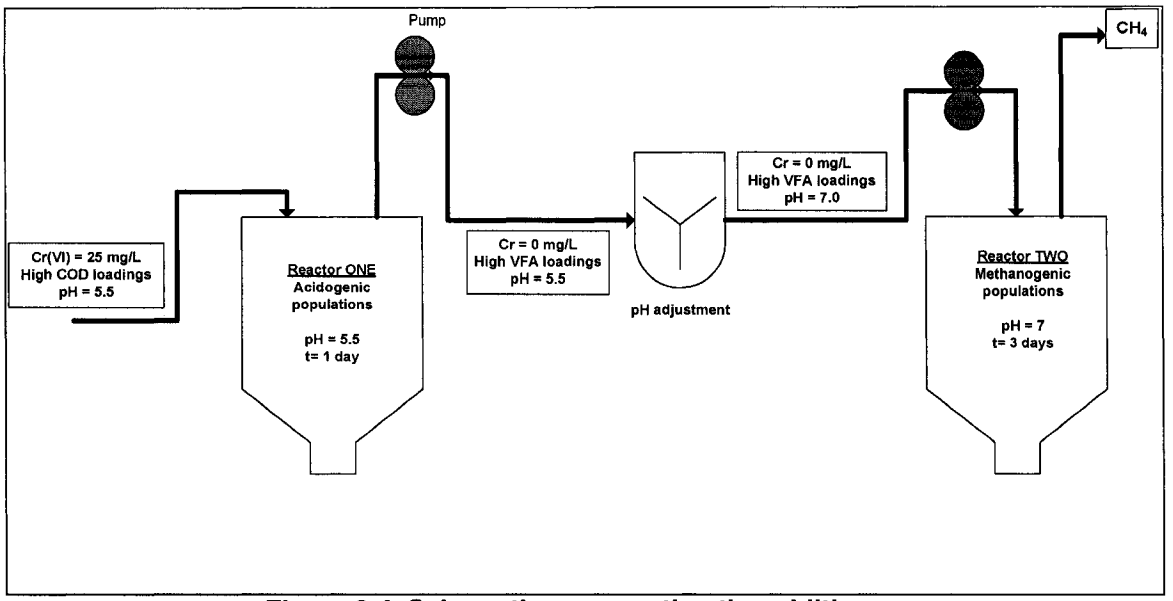

Figure 3-4. Schematic representing the addition sequence protocol for reactors one and two.

In addition, biogas production was measured by connecting the fermentation unit's condenser port to a gas collection unit. This unit estimates biogas production via water volume displacement. The volume of biogas produced by the reactor is equal to the volume of water displaced (Rozzi and Remigi 2004). The water was acidified to pH 2 with sulfuric acid in order to reduce the solubility of CO_2 gas in the barrier water solution. Methane is relatively insoluble in the water barrier solution.

After the 30 day phase optimization period, Cr(VI) was gradually introduced into the first reactor (phase I optimized). Different toxicity levels for chromium have been sited for anaerobic microorganisms in the literature; 0.078 mg/L Cr(VI) (Viamajala et al. 2004), 5 mg/L Cr(III) (Yu and Fang 2001), and 25 mg/L (Vance 2003) to mention a few. It is the norm to use a 10 fold concentration safety factor when exposing biota to toxic substances (Speece 1996). However, the results of

the batch study in this investigation show the cheese whey biomass to be quite resilient to chromium, and a level of 25 mg Cr(VI)/L was used in this study. Although typical industrial effluent concentrations for Cr(VI) ranged between 2 to 28.2 mg Cr(VI)/L (Adhoum et al. 2004, Selvaraj et al. 2003, Rana et al. 2004, Sapari et al. 1996), and in fact would be further diluted when mixed with an organic-containing waste stream, a level of 25 mg Cr(VI)/L was used in order to test the efficacy and resilience of the system at higher than expected levels of chromium.

Hexavalent chromium was introduced into reactor one at an initial concentration of 0.1 mg/L on day 1. Concentrations were gradually increased by adding feedstocks of 1, 5, and 12.5 mg Cr(VI)/L over the following three days. From day 5 onward the Cr(VI) concentration was fixed at 25 mg/L in the feedstock for reactor one. This was done to prevent an initial shock load of Cr(VI) to the system since non-acclimated biomass was used. A level of 1 mg Cr/L (which is about 100x the detection limit for the chromium determination method used) was chosen as the critical level of chromium in the effluent of reactor one. Although this chosen level was somewhat arbitrary it corresponds to about a 96% removal efficiency, and it was decided that a 95% or better removal for chromium was desirable. The treated liquid phase of reactor one was used as the feedstock for reactor two where methane production will be optimally maintained. Reactor one had the following cycling periods; filling (1 minute), reacting (22.5 h), settling (4 minutes), and draw and idle (1.5 h); whereas, the cycling periods for reactor two

were as follows: filling (1 minute), reacting (70.5 h), settling (4 minutes), and draw and idle (1.5 h).

The ASBR experiment was performed in duplicate. For reactor one, each duplicate run involved a 30-day acclimation period where biomass received daily additions of Cr-free simulated wastewater containing sucrose as the substrate, followed by a 42-day period of daily additions of Cr(VI)-containing simulated wastewater with sucrose. For reactor two, each duplicate run involved a 30-day acclimation period where biomass received additions of acetate-containing wastewater every three days, followed by an 84-day period where biomass received additions of Cr-free, VFA enriched influent (which was the effluent from reactor one derived from Cr(VI)-containing simulated wastewater) every three days (Figure 3-2).

3.4.5 Hexavalent Chromium Removal Using Biomass and Biomass Supernatant Components

Even after a one hour settling time, the settled cheese whey biomass is essentially comprised of a semi-solid biomass granule phase and a liquid phase with at least 25% water. The liquid phase or supernatant contains soluble and suspended organic material. In order to determine the Cr(VI) reduction or removal potential of the liquid and solid phases, a simple experiment was performed where supernatant, supernatant filtered through 0.45 μm filter, biomass granules washed with distilled water, and unwashed biomass granules

were placed in contact with Cr(VI) at 35 °C for 24 hours. All mixtures except for the supernatant filtered through the 0.45 μm filter, were filtered and analyzed for Cr(VI). Total chromium determination was performed on unfiltered aliquots. A procedural blank with Cr(VI) and distilled water was also prepared. The biomass granules were washed five times with distilled water for the washed biomass test. The biomass used was phase I optimized cheese whey biomass. The supernatant tests were prepared by adding 1 mL of a 750 mg Cr(VI)/L stock solution (pH 5.5) to 19 mL of supernatant. The biomass test was prepared by adding 8 mL of the same stock solution to 12 mL of settled biomass.

3.4.6 Speciation of Chromium Sorbed to Biomass

In order to determine the fate and speciation of sorbed Cr(VI), a selective sequential extraction was conducted on the biomass from reactor one at the end of the ASBR experiment. The fractions are categorized into six groups (Mulligan 1998): soluble, exchangeable, carbonate, (hydr)oxide, organic and residual. For each fraction, a specific extraction step was conducted. Table 3-3 shows the sequential extraction steps involved. Each extraction step fraction was collected and analyzed for chromium. The percentage extracted was based on the overall concentration of chromium in the biomass, which was obtained by concentrated acid cold digestion. The biomass was digested in polypropylene tubes with 6N nitric acid by shaking at 100 rpm on an orbital shaker until complete dissolution of biomass occurred.

Table 3-3. Steps for the selective sequential extraction experiment (Mulligan 1998).

Steps	Chemical Reagents	Fraction
1	Extraction of Cr by 15 mL of distilled water for 24 hrs.	Soluble
2	Extraction of Cr with 8 mL of 1 M magnesium chloride (pH 7) for 1 hour.	Exchangeable
3	Extraction of Cr with 8 mL of 1M sodium acetate adjusted to pH 5 with acetic acid for 5 hours.	Carbonates
4	Extraction with 20 mL of 0.04 M hydroxylamine hydrochloride in 25% (v/v) acetic acid pH 2.5 at 96 °C for 6 hours.	Oxides and hydroxides
5	Extraction with 3 mL of 0.02 M nitric acid and 5 mL of 30% hydrogen peroxide (pH 2) for 2 hours at 85 °C, followed by 3 mL of 30% hydrogen peroxide (pH 2) for 3 hours at 85 °C and then 5 mL of 3.2 M ammonium acetate in 20% (v/v) nitric acid diluted to 20 mL at room temperature for 30 minutes with distilled water.	Organic matter
6	Digestion for 3 hours at 90 °C with 25 mL of dilute aqua regia (5 mL of hydrochloric acid, 20 mL of nitric acid and 75 mL of distilled water).	Residual fraction

3.4.7 Leaching Test

A leaching test was conducted on chromium-contaminated spent biomass from reactor one using a standard leaching solution. Two leaching tests were performed: one on wet settled biomass and the other on the same biomass that was air-dried. The standard leaching solution was prepared by first adding 5.7 mL of glacial acetic acid to 500 mL of distilled water, followed by the addition of 64.3 mL of 1N sodium hydroxide; which was then diluted to one liter. A final pH value of 4.9 was measured for the leaching solution (EPA 1992).

Dried and settled wet biomass (2 grams each) were each placed in a 50 mL polypropylene tube to which 45 mL of the above standard leaching solution was added. Tubes, in triplicate, were placed on an orbital shaker, and shaken at 70 rpm for a period of five months. In the first week, 1 mL aliquots were taken daily for chromium determination. Subsequent 1 mL samples were taken every seven days for the remaining period.

Chapter Four

RESULTS and DISCUSSION

The results from both batch and ASBR experiments are presented in this chapter. The results for CO_2 and CH_4 composition in the biogas are provided as percentage pure gas, based on peak heights, obtained for either serum bottle or gas collection unit head space, compared to pure carbon dioxide and methane gas samples, as determined by gas chromatography. Changes in COD and VFA content in the reactor liquid phase were calculated with reference to initial or control values. Chromium removal was determined by the difference between initial and final concentrations in supernatants or reactor liquid phases.

4.1 Batch Experiments

Phase optimized biomass was prepared so as to take advantage of optimum conditions for acidogenesis or VFA production in reactor one as well as methanogenesis in reactor two. The lower pH of reactor one would also tend to favor reduction of Cr(VI) to Cr(III) since this reduction increases with decreasing pH (Krishnani et al. 2004). However, the goal is not to completely eliminate methanogenesis in reactor one. It is desirable to maintain some methanogenic activity so that VFAs, which are removed during methane production, will not reach levels where their further production by acidogenesis is inhibited; which would be the case if their concentration is allowed to build up beyond a critical

level (Andrews 1989). Fortunately, it is impossible to completely separate the anaerobic biodegradation process into two separate phases (acidogenic and methanogenic), the term phase-optimized is based on the relative composition of the biogas with respect to CO_2 and CH_4 as measured by gas chromatography. Higher ratios of CH_4/CO_2 in a phase II optimized biomass, when compared to phase I optimized biomass, are indicative of enhanced methanogenic activity. Table 4-1 gives the CO_2 and CH_4 content of the biogas for each phase optimized system as well as the CH_4/CO_2 ratio after a 24 h period (section 3.2.2).

Table 4-1. Carbon dioxide and methane gas content in the phase optimized biomass after a 24h period.

Biomass	CO_2	CH_4	CH_4/CO_2
Non-optimized	42%	40%	0.95
Phase I optimized	51%	30%	0.59
Phase II optimized	30%	55%	1.83

As stated earlier, the amount of water in the settled biomass is an important consideration with respect to final estimated chromium levels since granular water will dilute the added chromium solution. By estimating the amount of granular water, chromium concentration can be adjusted to take this water into consideration. The concentrations of Cr(VI) referred to in this study were based on total water content which included estimated average granular water volume.

Results from 10 replicates showed that for each 12 mL of settled biomass there was an average of 3.0 ±0.2 mL of free or bulk water. Therefore, for each 2 mL of

added chromium solution, there was 12 mL of liquid phase (section 3.4.2 for serum bottle composition) yielding a 7-fold dilution of the original added Cr(VI) solution.

4.1.1 Chromium Solubility Test

Since hexavalent chromium will be introduced into an anaerobic reactor maintained at pH 5.5, and it is also expected that some of the hexavalent chromium will be reduced to the trivalent species, a simple test was performed to investigate the feasibility of using Cr(III) in the sorption study, given that it is known to be quite insoluble at pH values above 5 (section 3.4.2).

The results of incubating and shaking a 500 mg Cr(III)/L solution, adjusted pH 5.5, for 24 hours indicated that most if not all of the Cr(III) was removed from the supernatant through precipitation. A thick milky precipitate was observed after pH adjustment. Table 4-2 shows the concentrations measured in the supernatant after centrifugation and filtration.

Table 4-2. Initial Cr(III) and Cr(VI) concentrations before and after pH Adjustment.

Initial values of Cr(III) before pH adjustment (mg/L)	Initial values of Cr(VI) before pH adjustment (mg/L)	Measured concentration of Cr(III) after pH adjustment (mg/L)	Measured concentration of Cr(VI) after pH adjustment (mg/L)
487	493	2	491
503	506	2	507
490	510	4	500
Avg. 493	**Avg. 503**	**Avg. 2.7**	**Avg. 499**

The results clearly demonstrate the impracticality of using Cr(III) in the batch study with pHs greater than 5. Again it should be recalled that Cr(VI) is being introduced into the bioreactor. The precipitation of Cr(III) under the bioreactor pH conditions is however of some value in elucidating the method of chromium removal, since as was mentioned earlier, hexavalent chromium reduction to trivalent chromium is highly likely under the prevailing conditions of the anaerobic bioreactor.

4.1.2 Hexavalent Chromium Concentration Study

Serum bottle tests were performed in duplicate. As stated previously, each bottle contained the following: 12 mL of settled biomass + 8 mL of wastewater + 2 mL of Cr(VI) solution + 1 mL of pH adjustment solution. The duration of the test was two days. Figure 4-1 illustrates the effect of Cr(VI) concentration on the CH_4/CO_2 ratio of the headspace biogas after a two day incubation period. The initial CH_4/CO_2 ratio for each biomass closely matches that obtained for the respective stock biomass. The phase II optimized biomass, which had the highest initial CH_4/CO_2 ratio, exhibited the greatest reduction in this ratio with increasing Cr(VI) exposure levels. Comparison of the slopes between concentration intervals for phase I and phase II optimized biomass might imply that methanogenesis was more affected in the phase II optimized biomass when compared to the phase I and non-optimized biomass. Although the initial CH_4/CO_2 ratios for the phase I and non-optimized biomass were different, their behavior, as far as the slopes between concentration intervals, was similar. One

thing can be said for certain is that methanogenic activity in phase II biomass was completely halted at a Cr(VI) exposure level of 223 mg/L, whereas complete cessation of methanogenesis occurred at a Cr(VI) exposure level of 446 mg/L for phase I optimized biomass.

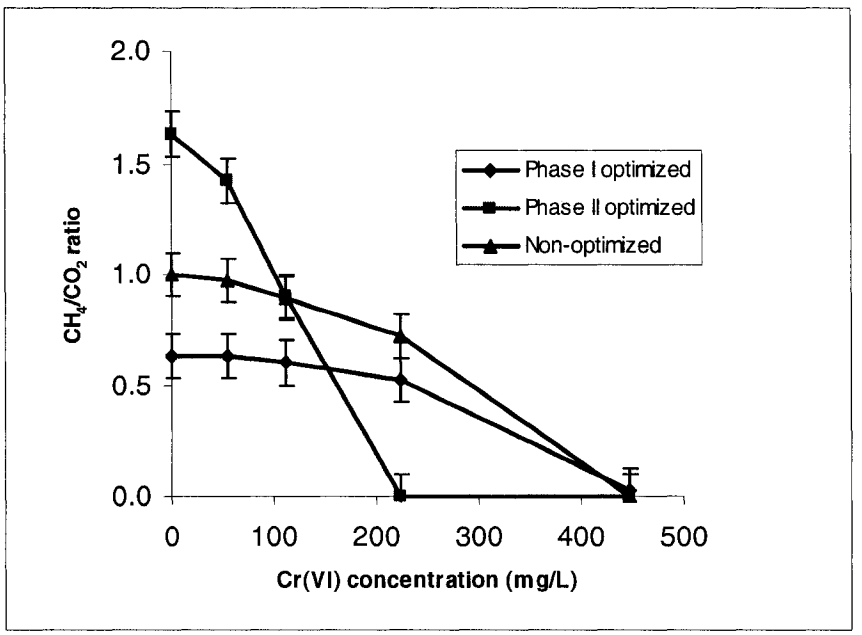

Figure 4-1 Effect of Cr(VI) concentration on the CH_4/CO_2 ratio after a two day incubation period.

These results indicate that methanogens in the phase II optimized biomass were more sensitive to Cr(VI) exposure than their phase I optimized counterparts. This naturally leads to the following question: Why was methanogenic activity completely inhibited in the phase II optimized system and not in the phase I optimized systems at a concentration of 223 mg Cr(VI)/L?

One plausible explanation may be elucidated in terms of the difference in the phase I and phase II anaerobic biomass granules. Figure 4-2 is a photograph of magnified anaerobic cheese whey biomass granules that were the original biomass stock used in this study. The photograph clearly shows two distinct granules types; a larger, light colored granule and a smaller dark colored one. It was observed in this study that phase optimized granules was also of two types with respect to size and color. Phase I optimized biomass was composed of light colored, slightly larger granules: whereas, phase II optimized biomass was composed of darker slightly smaller granules (Figure 4-3).

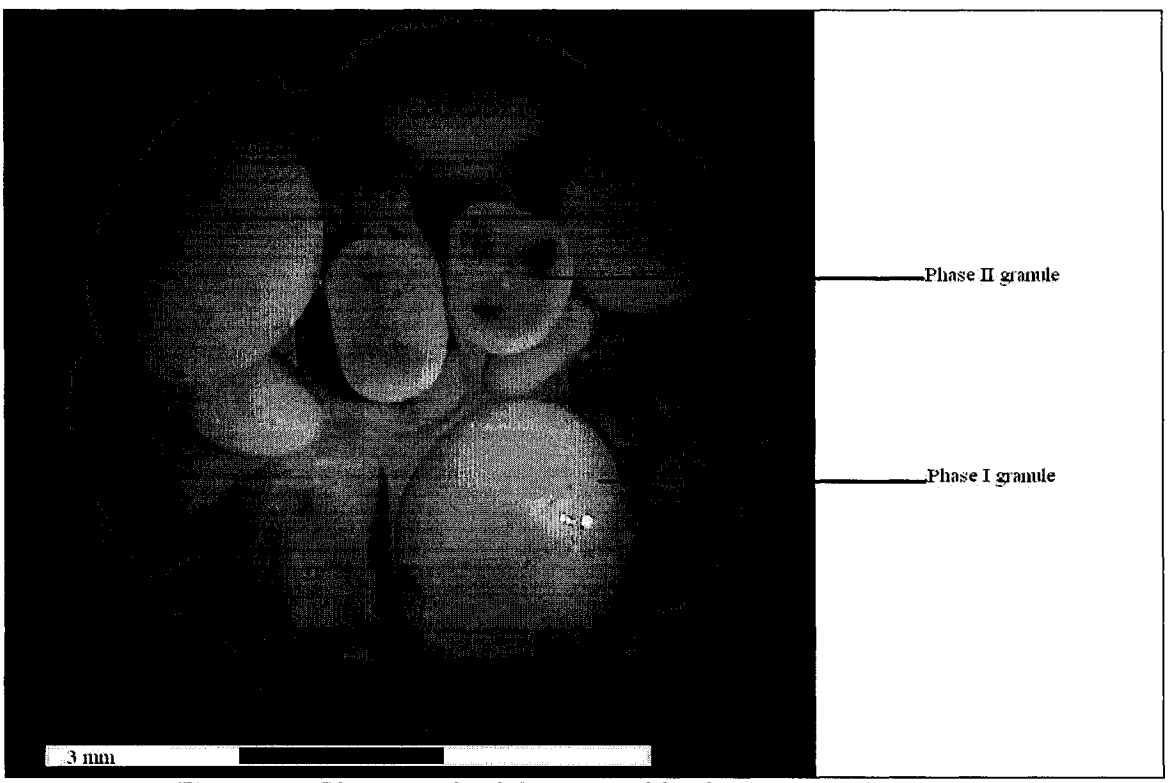

Figure 4-2. Photograph of the anaerobic cheese whey biomass used in this study (50x magnification).

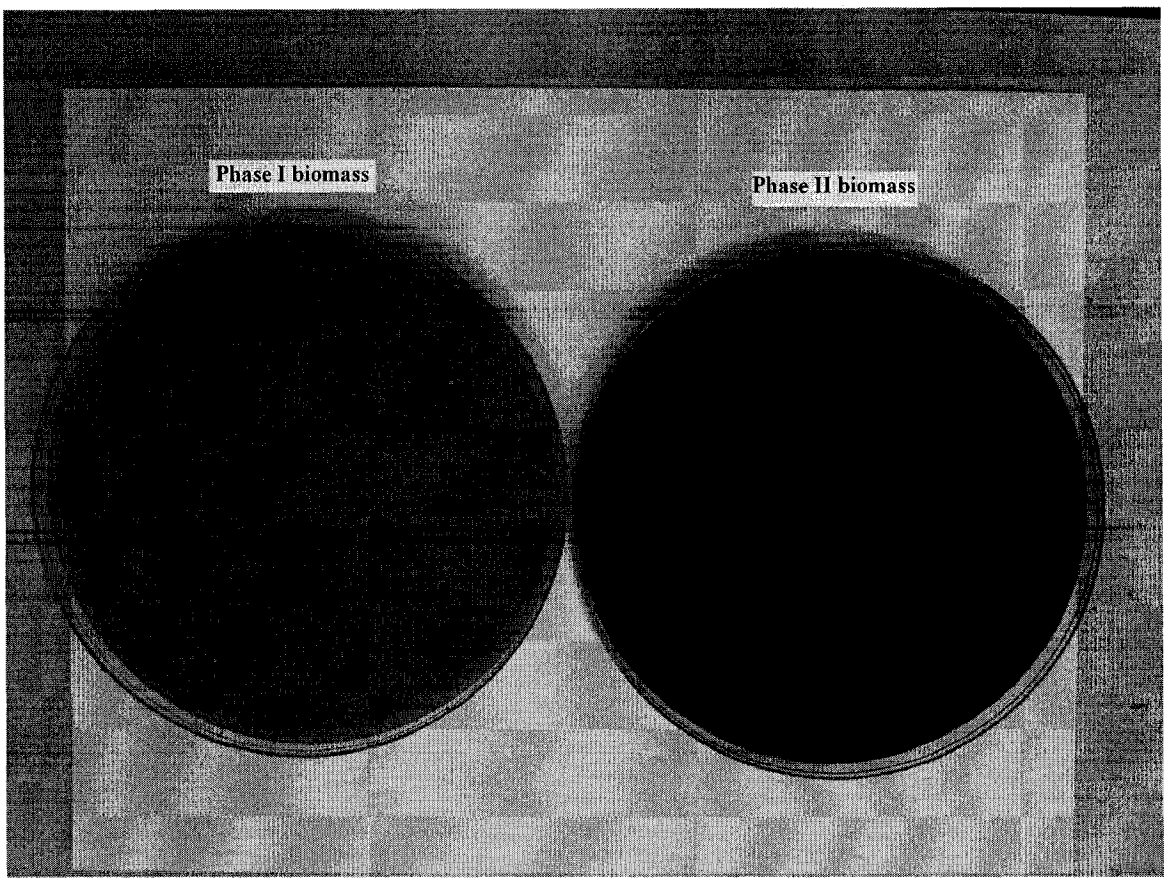

Figure 4-3. Phase I and phase II optimized biomass.

Studies have attempted to elucidate the finer structure of anaerobic biomass granules and have shown that the bacterial populations in these granules are arranged in somewhat distinct layers (Fang et al. 1994). These granules are comprised of a dark colored inner core containing methanogens. This core is surrounded by a layer of a mixed population of acidogenic, synthropic and methanogenic bacteria. The outer core consists primarily of acidogenic bacteria with some synthropic and to a lesser extent methanogenic bacterial populations (Figure 4-4).

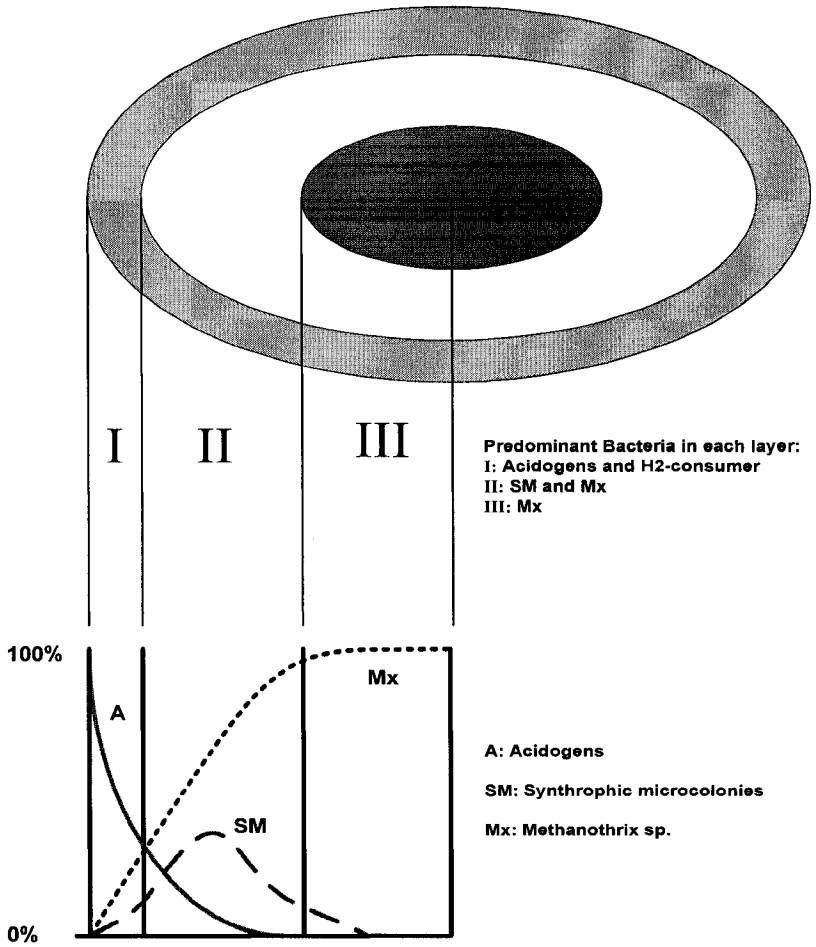

Figure 4-4. Proposed layered structure and bacterial composition for the granules treating soluble carbohydrates (Fang et al. 1994).

The above photographs (Figures 4-2 and 4-3) indicate that the smaller darker granules in the phase II optimized biomass may be comprised predominantly of the inner core methanogens as described in Figure 4-4; whereas, those in the phase I optimized biomass may contain an inner core of methanogens and outer layers of acidogens. The answer to our question may lie in the preceding granule structure composition. In the phase I optimized biomass the outer bacterial layers may in fact protect the inner methanogenic core from Cr(VI)

toxicity. Sorption and/or reduction of Cr(VI) could take place at this outer layer thereby reducing the effective concentration of Cr(VI) at the inner core relative to the concentration in the bulk solution. In the phase II optimized biomass the outer, lighter colored layers were apparently absent, and the methanogens would have been exposed to a relatively higher and thus more toxic level of Cr(VI) than the phase I optimized biomass given the exact same concentration in the bulk fluid. Thus even though the phase I optimized biomass still has some methanogenic activity, the methanogens may be surrounded, and thus protected from the toxic effects of Cr(VI), by an outer layer of acidogenic bacterial populations. In the phase II optimized biomass the methanogens may have less acidogenic bacteria surrounding them and as a result may be more exposed to Cr(VI) than their phase I optimized counterparts. The apparent greater resistance to Cr(VI) by the phase I optimized biomass with respect to methanogenesis is an important result since it is desirable to maintain at least some methanogenic activity, for as long as possible, and at some minimum critical level in reactor one of the system in order to facilitate VFA production. If VFAs produced during the acidogenic phase are not processed to some extent by the methanogenic population they can build up to a significant level so as to suppress further acidogenic biodegradation (Andrews 1989).

Figure 4-5 illustrates the effect of increasing Cr(VI) concentrations on COD removal in phase I and phase II optimized as well as non-optimized biomass. Results for phase I and non-optimized biomass show little or no difference for

exposure levels of Cr(VI) up to 223 mg/L. However, phase II optimized biomass display a relatively marked decrease in COD reduction with increasing Cr(VI) exposure levels if a comparison of the slopes between concentration intervals is made. At an exposure level of 223 mg Cr(VI)/L, COD reduction was 19% compared to 28.3% for the control biomass: thus, there was about a 32% reduction in the phase II optimized biomass's ability to reduce COD.

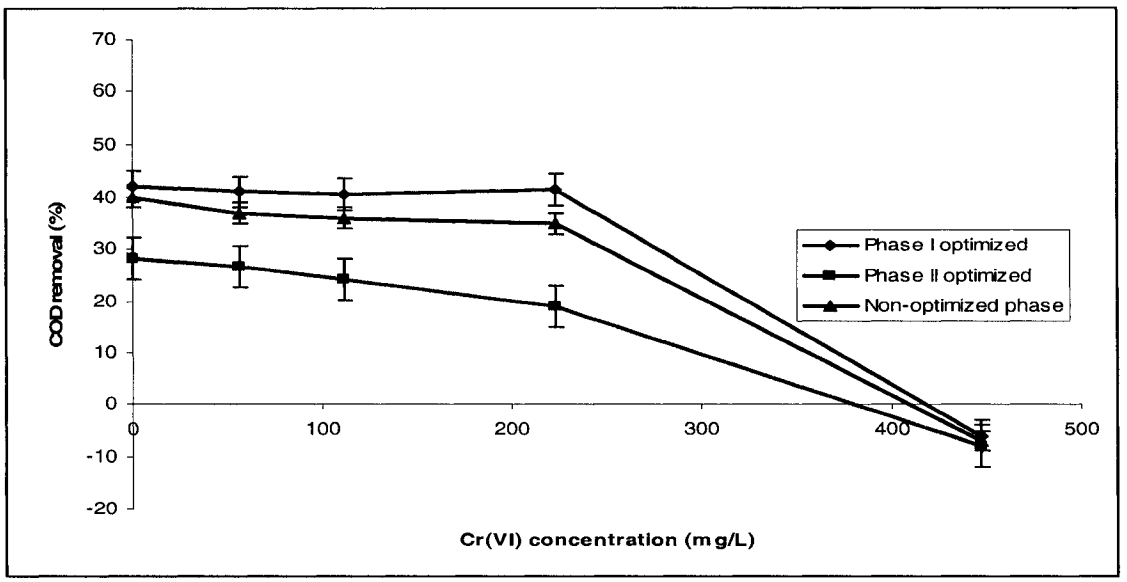

Figure 4-5. Effect of Cr(VI) on COD removal for the three phases.

At 446 mg Cr(VI)/L Figure 4-5 shows a complete cessation of COD reduction. However, at concentration of 446 mg Cr(VI)/L the measured soluble COD was actually slightly greater than the initial soluble COD values, resulting in negative COD reduction. The average increase in COD values at concentrations of 446 mg Cr(VI)/L was about 6% for phase I and 8% for phase II optimized systems. Since all tests were done in duplicate and all COD values were consistently above initial COD levels random experimental error seems unlikely. One

explanation for this consistent slight relative increase in soluble COD may be the occurrence of cell lysis, or rupture, due to the toxic levels of Cr(VI). When cells rupture intracellular organic material spills out into the surrounding bulk fluid thereby increasing measured COD levels.

The increases in COD levels may however be an artifact resulting from the COD test method used in the analysis. The test method used was a closed reflux technique which uses potassium dichromate, a hexavalent chromium compound, to oxidize organic matter in the test solution. The hexavalent chromium is concomitantly reduced to trivalent chromium which forms a colored complex under test conditions. The degree of light absorption due to this colored complex is correlated with original levels of chemically oxidizable organic matter, and forms the basis for this COD test. It is reasonable to assume that adding increasing levels of hexavalent chromium (potassium chromate in this case) may artificially increase COD levels relative to control samples since the chromate anion may also oxidize organic matter and produce trivalent chromium. Thus, a control test sample, with no exposure to hexavalent chromium, when mixed with the hexavalent chromium-containing COD test solution, would contain much less hexavalent chromium than a test sample which already contained hexavalent chromium prior to adding the COD test solution. This becomes an important consideration if confidence in the COD values is to be maintained, since all exposure test samples contained potassium chromate as the hexavalent chromium species to some degree.

In order to determine if the increase in COD relative to the control levels were due to cell lysis, or an artifact due to elevated levels of Cr(VI), a simple test was performed. A triplicate COD test analysis was performed using potassium hydrogen phthalate (KHP). KHP is the theoretical COD test compound which is oxidized by dichromate under the test conditions. Two milliliters of a 500 mg/L COD phthalate equivalent solution were added to each of six COD vials. To three control vials, 0.5 mL of distilled water was added, and to the remaining three test vials, 0.5 mL each of a 5000 mg Cr(VI)/L. A theoretical COD of 400 mg/L for each vial was thus obtained. The hexavalent chromium level in the test vial's 2.5 mL added aliquot, in excess of the test vial levels, was thus 1000 mg Cr(VI)/L.

After processing, no significant difference in measured COD levels between the control and test vials was observed. Control vials, with no additional hexavalent chromium had an average COD value of 410 mg/L ± 8%, and test vials, with additional hexavalent chromium, had an average COD value of 402 mg/L ± 9%. Therefore, it would appear that the elevated COD levels measured at the higher Cr(VI) exposure levels were not artifacts resulting from the closed reflux COD test method, and is most likely the result of cell death and lysis, whereby intracellular organic material is released into the surrounding liquid phase. Again it must be stressed that it is the lower concentration range that is of interest, and the data clearly show that at concentrations above 223 mg Cr(VI)/L there is an adverse effect with regards to COD removal. It can also be

said that the phase II optimized system was affected more than the phase I system as was also the case with respect to methanogenesis.

Batch test results indicated that the removal efficiency of the biomass (112 mL settled volume) for Cr(VI) at concentrations up to and including 446 mg Cr(VI)/L was 100%. These results validate the comparison of toxicity across the entire exposure range for Cr(VI). In other words, all of the toxic effect can be directly related to the amount of chromium sorbed to biomass granules, as well as to the initial chromium concentrations.

4.1.3 Hexavalent Chromium Toxicity to Cheese Whey Biomass Based on the Batch Study

Hexavalent chromium is known to be toxic to most living organisms due to its ability to oxidize organic matter, forming chromium-organic matter complexes. Cr(VI) is also known to cross-link DNA molecules resulting in damage to genetic material. It is considered a cytotoxic agent; that is, capable of causing cell death or disfunction (Bagchi et al. 2002, O'Brien et al. 2001). As far as the toxicity of Cr(VI) to the cheese whey biomass in the batch study is concerned, its toxic effect is most likely related to its ability to cause immediate oxidative damage to bacterial cells in the biomass granules. This could occur through damage of the bacterial cell membrane or uptake of Cr(VI) into the cytoplasm where oxidative damage could also occur (Ahalya et al. 2003, Gadd 1990).

The toxicity of any compound is determined using standard bioassay methods. In general, the methods involve administering a known dose of the toxicant to test organisms in order to determine any toxic effects. A median lethal dose, the LD50, or the dose that kills 50% of the test population, is then determined. However, when dealing with microorganisms the dosing methods of classical toxicology are not strictly applicable. Instead, the toxicological testing methods of aquatic toxicology are applied, whereby instead of an administered dose the concentration of the toxicant in the water phase is used to determine toxic levels.

In lethal, aquatic toxicity tests, the percent mortality of the test organisms versus the concentration of the toxicant in the water phase is used to statistically determine a lethal toxicity level. Standard toxicity levels or concentrations for any aquatic test organism are traditionally given as the concentration that kills 50% of the test population. This standardized toxicity concentration is known as the LC50 (EPA 1998). Non-lethal toxicity tests use a standard toxicity concentration known as the EC50, or the concentration of toxicant that affects 50% of the population. The effects, in the case of EC50 determinations, may be physiological, biochemical, or even behavioral. In addition, the IC50, the concentration that inhibits 50% of a quantifiable biological process, is also used (EPA 1998). The goal of indexing the toxicity of Cr(VI) is to assess the feasibility of a laboratory scale ASBR experiment by relating toxicity to sorption capacity of the biomass to Cr(VI). The batch test

studies were employed simply as a range finding tool to investigate the toxicity of Cr(VI) to the anaerobic biomass; especially at higher chromium loadings, which would eventually occur due to accumulation after repeated low level of Cr(VI) additions during the ASBR experiment.

A standard toxicity testing protocol could not be strictly applied to the biomass involved in this study since it contained a varied, mixed population of bacteria, as well as extra-cellular substances which could bind, and thus attenuate the exposure concentration of chromium. In order to quantify or index the overall toxicity of Cr(VI) to the cheese whey biomass, the relative COD removal can be used as a quantifiable toxicity indicator. In using the COD removal capacity, with respect to the control (0 mg Cr(VI)/L) for increasing Cr(VI) exposure levels an examination of the overall effect on both the acidogenic and methanogenic bacterial populations is possible.

The simplest graphical method used for determining LC50 is the linear log-interpolation method. This method is widely used for toxicity screening of bacterial populations, especially in aerobic unit operations (EPA 1998). Most populations of organisms exhibit a wide range of sensitivities that can span a few orders of magnitude, and in the linear log-interpolation method, the two closest data points on either side of the 50% effect or mortality ordinate are plotted against log of the toxicant exposure concentration. Thus, assuming that reduction in overall COD removal is due to a lethal toxicity effect, an LC50 can

be estimated for hexavalent chromium (EPA 1998). However, since it cannot be ascertained that the toxic effect, in this case an overall reduction in the capacity of the biomass to remove COD, is solely due to cell death, the toxicity to Cr(VI) can also be expressed as an IC50. Figure 4-6 illustrates the linear log-interpolation method for determining the IC50 for each biomass system. Both phase I and non-optimized biomass showed almost the same sensitivity to Cr(VI) with respect to COD removal, having IC50 values of 309 and 302 mg Cr(VI)/L. However, the phase II optimized biomass had an IC50 value of 263 mg Cr(VI)/L, indicating increased sensitivity to hexavalent chromium. Since it is known from the CH_4/CO_2 ratios versus Cr(VI) exposure levels that methanogenesis was most likely more affected than acidogenesis, the increased sensitivity as far as COD removal for the phase II optimized biomass was related to an increased inability of methanogens to process substrate; indeed, methanogenic activity was completely halted at a Cr(VI) exposure level of 223 mg/L.

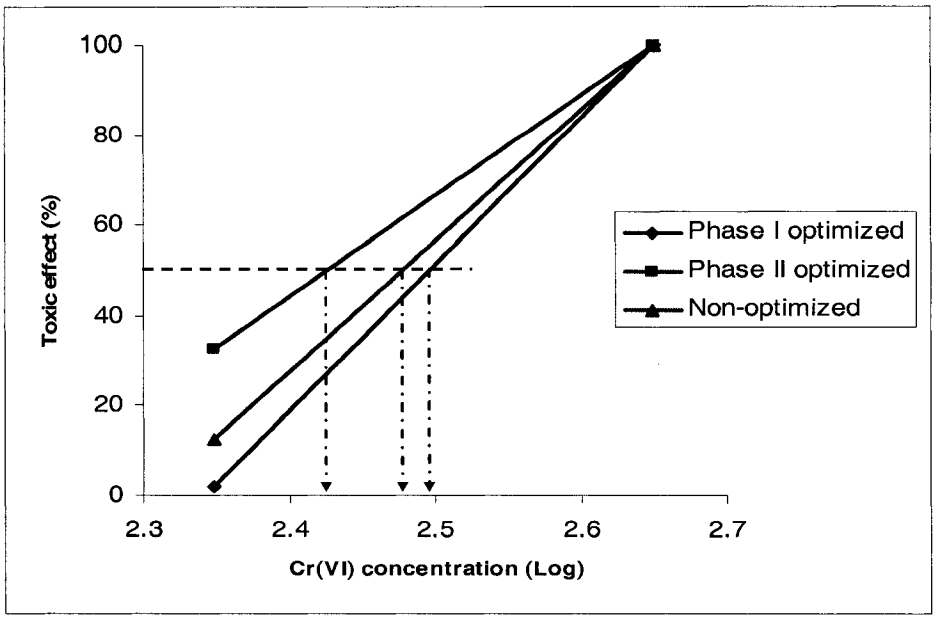

Figure 4-6. IC50 determination for the three biomass systems with respect to the percent reduction in COD removal.

As discussed earlier (section 4.1.2) the biomass sorbs essentially all the added Cr(VI). In addition, the equilibrium study prelude to sorption experiments showed that the maximum sorption of Cr(VI) occurred within 15 minutes (section 4.2.3.1). In addition, as mentioned previously, the aim is to correlate or compare toxicity with sorption capacity, thus applying the concentration of toxicant versus percent of the test population affected methodology is not appropriate for this purposes. Since the added Cr(VI) is rapidly and completely sorbed to the biomass granules, the added Cr(VI) is tantamount to an administered dose if the biomass granule is considered as an "organism". The granule can be considered a multicellular system comprised of different bacterial populations working in tandem. The biomass granule functions much as a multicellular organism, where the metabolic byproducts of certain biochemical reactions, associated with particular cell populations, can affect the

biological activity of other cell populations in the system. For example, the rate of production of VFAs by acidogenic bacterial populations of the granule can be affected by methanogenic bacterial population activity. Thus using an index that correlates toxicity with an effective "dose" seems reasonable under these circumstances.

Since the settled volume of biomass (12 mL) and the amount of Cr(VI) added in the batch test experiments is known, and it was shown that at the highest Cr(VI) exposure level 100% of the added Cr(VI) is removed or sorbed by all three biomass optimization types, the IC50 values can be effectively transformed to ID50 values, or a "dose" that inhibits overall soluble COD removal by 50%. Although no other researchers have used ID50 values in conjunction with biomass granules, the data and the above considerations justify this novel approach; especially since the use of ID50 values allows for the development of models which can compare sorption capacity and toxicity for the biomass under investigation.

The respective ID50 values for phase I, non-optimized, and phase II biomass are: 283, 278, and 241 mg Cr(VI)/L settled biomass. It must be stressed that the IC50 (and ID50) values reported and referred to in this study are not to be taken in a strict toxicological sense, but serve as practical indices of toxicity. It should be stated at this point that the classical standard method for determining the toxicity of heavy metals or other organic toxicants to methanogens in anaerobic

systems is the specific methanogenic activity (SMA) test (Soto et al. 1993). In general, this test involves feeding anaerobic sludge or biomass a mixture of VFAs (such as acetic, butyric, and propionic acid) as the substrate or COD, and determining the methane production rate. The activity is usually expressed as g COD·g VSS^{-1}·d^{-1} (Soto et al. 1993). The reduction of methane production per COD removed per time relative to the control reactor is calculated and an inhibition index such as the $C_{I,50}$, or the concentration of toxicant that inhibits the SMA by 50% for example, is determined (Fang and Chan 1997). Although the conditions in the batch test studies favored the application of the SMA test method, no reliable method for determining biogas pressure in the sealed serum bottles was available. The most reliable methods are to use either pressure transducers or respirometers in conjunction with gas chromatography to determine biogas production and methane content (Ince et al. 1995, Cimochowicz-Rybicka and Kocwa-Haluch 1999). As neither instrumental options was available, a syringe displacement technique (Fang and Chan 1997) to determine relative biogas production between controls and test serum bottles was used. The syringe needle penetrates the rubber septum seal and the pressurized headspace will cause the syringe plunger to rise. However, attempts to apply this technique resulted in large experimental errors. The large discrepancy between errors encountered (>75%) within a replicate series for gas production and those obtained for COD removal or content of methane (<15%) indicated the unsuitability of the results with respect to analysis according to the SMA method.

However, it should be stressed at this point that the focus here was the relative toxicity of Cr(VI) to phase I and phase II optimized biomass. Even if methane production rate was determined, relative to COD removal, the initial COD or substrate was different for each.

Phase I optimized biomass received sucrose and was initially prepared to simulate biomass in reactor one of a two-phase ASBR system, whereas, phase II optimized biomass were initially prepared to simulated the biomass in reactor two. Since reactor two would receive no chromium, the SMA test is not applicable. Indeed the only reason for investigating the toxicity of chromium to phase II optimized biomass is purely academic since the original goal is to maintain reactor two containing a phase II optimized biomass under conditions of no or negligible chromium. Thus it is only the phase I optimized biomass that is of interest as far as hexavalent chromium toxicity is concerned. The relative reduction in COD removal was in the final analysis considered a good indicator for a general or overriding toxicity index to gage metabolic activity under Cr(VI) loading in preparation for the ASBR experiment, since it incorporates the combination of COD removal by both acidogenic and methanogenic bacteria. Thus, the ID50 value of 283 mg Cr(VI)/L settled biomass obtained for phase I optimized biomass in conjunction with the sorption capacity of the cheese whey biomass for Cr(VI) gives us some initial data with which to assess the potential for chromium removal using a two-phase ASBR system.

4.1.4 Sorption Study

4.1.4.1 Equilibrium Study

Equilibrium experiments are required to determine when equilibrium between dissolved and sorbed solute has taken place. Figure 4-7 shows the sorbed or removed Cr(VI) with time. Control Cr(VI) tubes, without biomass, showed no significant interfering sorption due to the polypropylene tubes, and thus all measured sorption was mediated by the biomass. Cr(III) was not detected in the supernatants. The difference between initial Cr(VI) and final Cr(VI) in the supernatant was accounted for by determining total chromium on the biomass for each relevant supernatant. The average amount of chromium sorbed based on digestion of the biomass was within 12% of the estimated sorption based on differences between initial and final Cr(VI) supernatant concentrations.

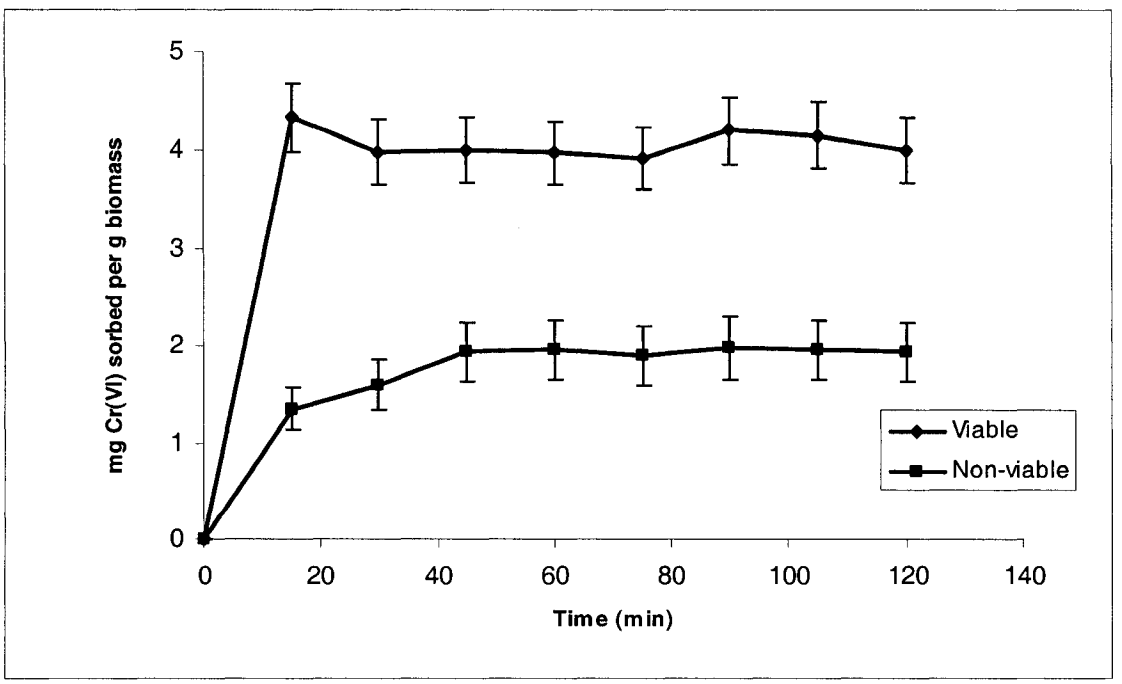

Figure 4-7 Removal of Cr(VI) versus time for viable and non-viable biomass.

This experiment showed that Cr(VI) is maximally sorbed within 15 minutes for viable biomass and within 45 minutes for the non-viable biomass. However, it is difficult to discern why the viable biomass apparently achieved equilibrium sooner than the non-viable biomass. One possible reason is that the dried biomass required some time to hydrate and expand until Cr(VI) could effectively penetrate the granule. It is the time to reach equilibrium that is important in this experiment rather than the amount of Cr(VI) sorbed since different initial chromium concentrations and biomass weights were used (which were based on dry weight for non-viable and wet weight for viable biomass). The relative sorption capacities of these two are discussed in the following section.

4.1.4.2 Sorption Isotherm for Biomass

The sorption isotherm for the viable biomass best fit the Langmuir model (R^2=0.9933) when compared to the Freundlich (R^2=0.98) and the linear models (R^2=0.66). The data presented below are developed according to the Langmuir formulation. Figure 4-8 is a plot of experimental milligrams of Cr(VI) sorbed per gram of wet weight biomass as a function of equilibrium Cr(VI) concentration. The Langmuir data transformation plot is presented in Figure 4-9.

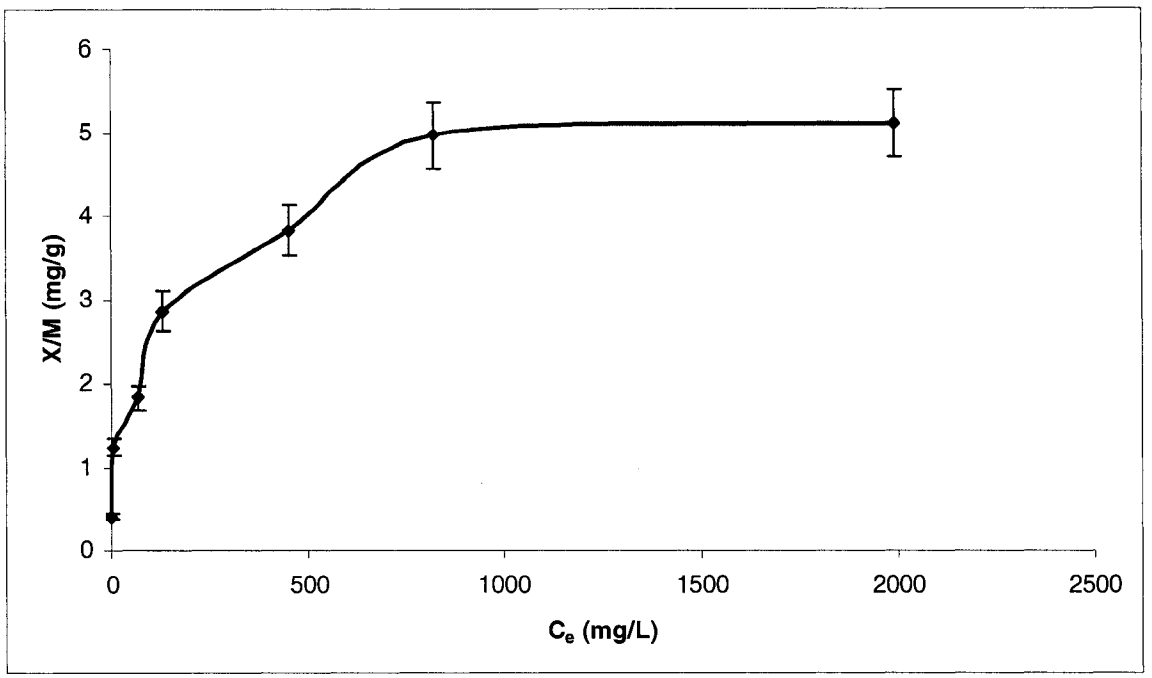

Figure 4-8 Plot of experimental Cr(VI) sorbed per gram of wet weight biomass as a function of equilibrium Cr(VI) concentration (X/M vs. C_e).

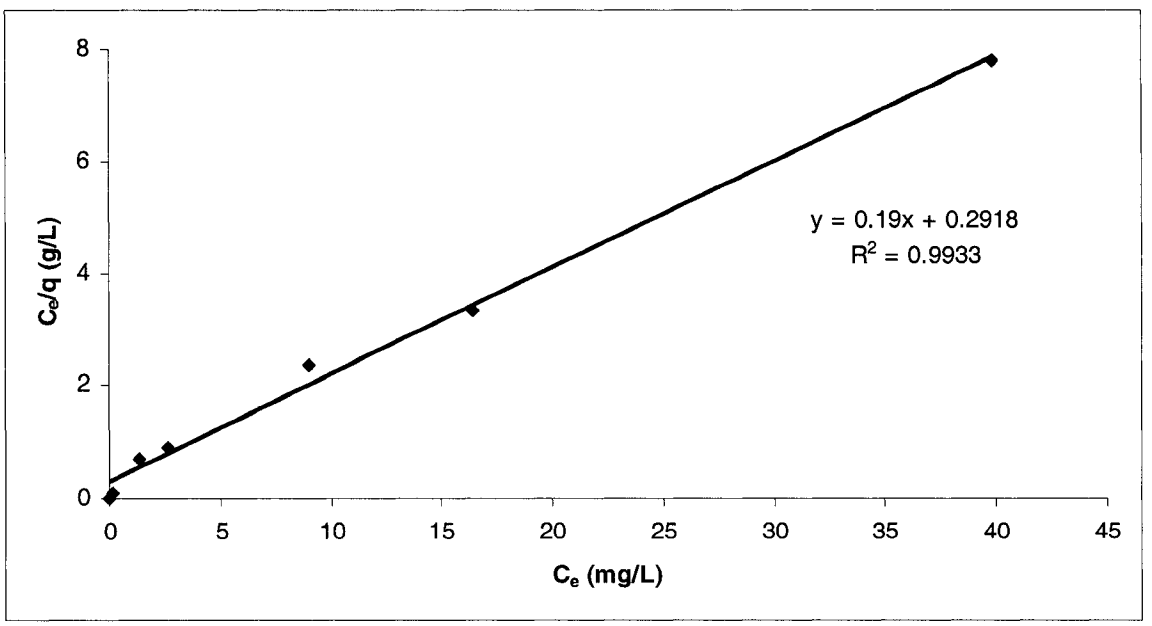

Figure 4-9. Isotherm data plotted according to the Langmuir model for adsorption for viable biomass.

Equation 4-1 represents the Langmuir equation derived from the above isotherm:

$$\frac{C_e}{q} = \frac{C_e}{5.26} + 0.2918 \qquad 4\text{-}1$$

Figure 4-10 shows the theoretical Langmuir sorption isotherm along with the experimental data points to visually compare the goodness of fit.

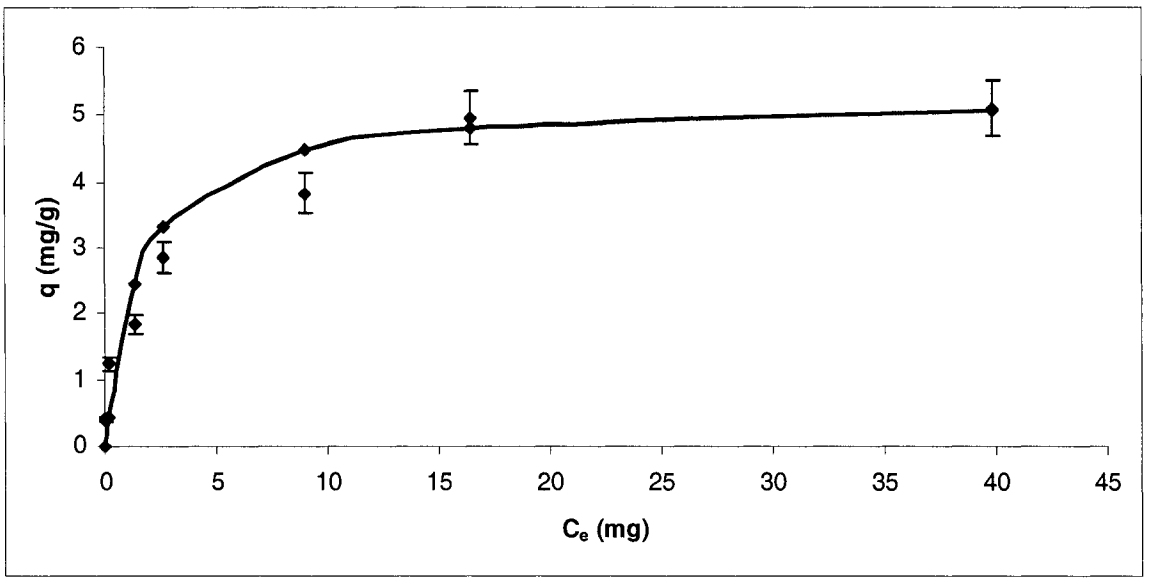

Figure 4-10. Theoretical Langmuir isotherm with experimental data points for viable biomass.

The theoretical maximum amount of Cr(VI) adsorbed per unit mass wet weight of biomass can be estimated using the Langmuir model. Viable biomass was found to have a q_{max} of 5.26 mg Cr(VI)/g wet weight.

Even though the topic of this investigation is the removal of Cr(VI) from waste streams using viable cheese whey biomass, while maintaining optimum

methane production, much interest as of late has focused on using non-viable heat treated biomass to remove heavy metals (Bai and Abraham 2003, Aksu et al. 2002). A sorption study using dried non-viable cheese whey biomass was also carried out in order to compare removal potential for Cr(VI) by both types. Figure 4-11 is a plot of Cr(VI) sorbed per gram of dry weight non-viable biomass as a function of equilibrium Cr(VI) concentration. The sorption isotherm for the non-viable biomass fitted the Langmuir model (R^2=0.9987) much better than the Freundlich (R^2=0.79) or the linear models (R^2=0.39) as shown in Figure 4-12.

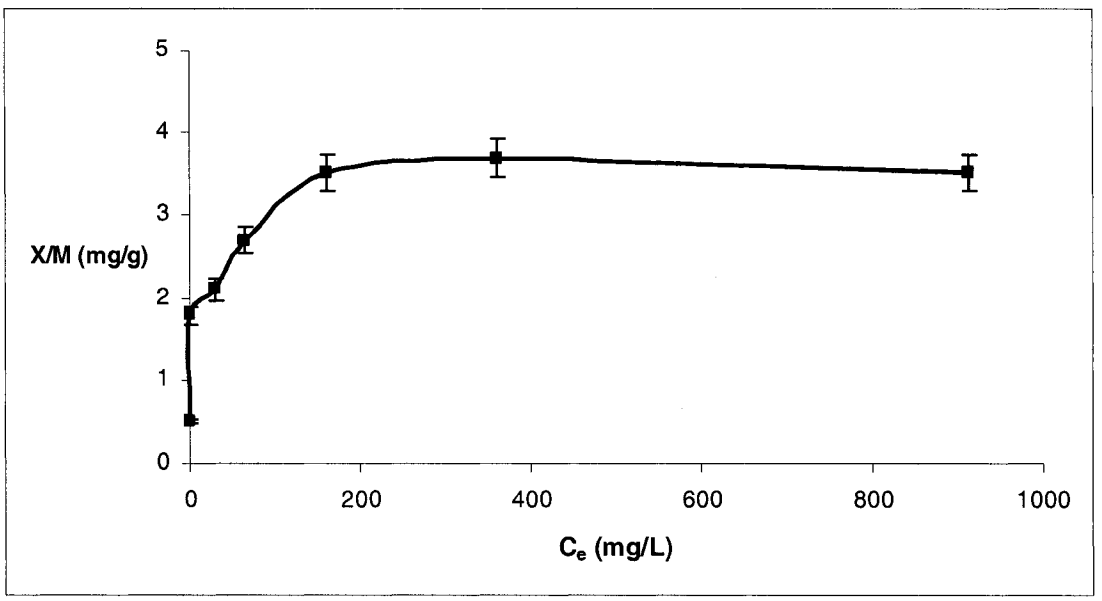

Figure 4-11 Plot of experimental Cr(VI) sorbed per gram of dry weight biomass as a function of equilibrium Cr(VI) concentration (X/M vs. C_e).

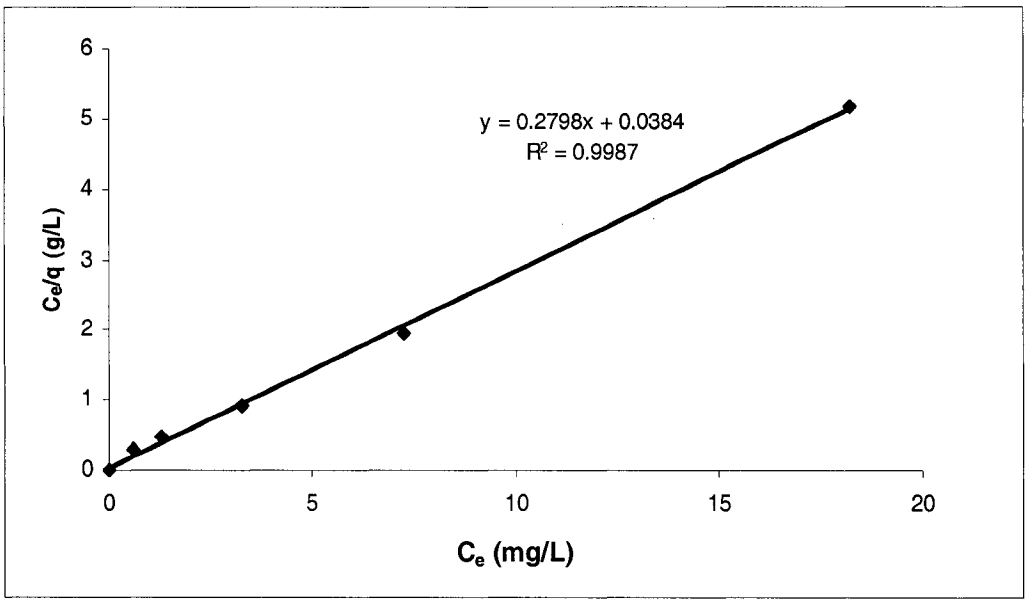

Figure 4-12. Isotherm data plotted according to the Langmuir model for adsorption for non-viable biomass.

Equation 4-3 represents the Langmuir equation:

$$\frac{C_e}{q} = \frac{C_e}{3.57} + 0.2798 \qquad 4\text{-}3$$

Figures 4-13 shows the theoretical Langmuir sorption isotherm along with the experimental data points to visually compare the goodness of fit.

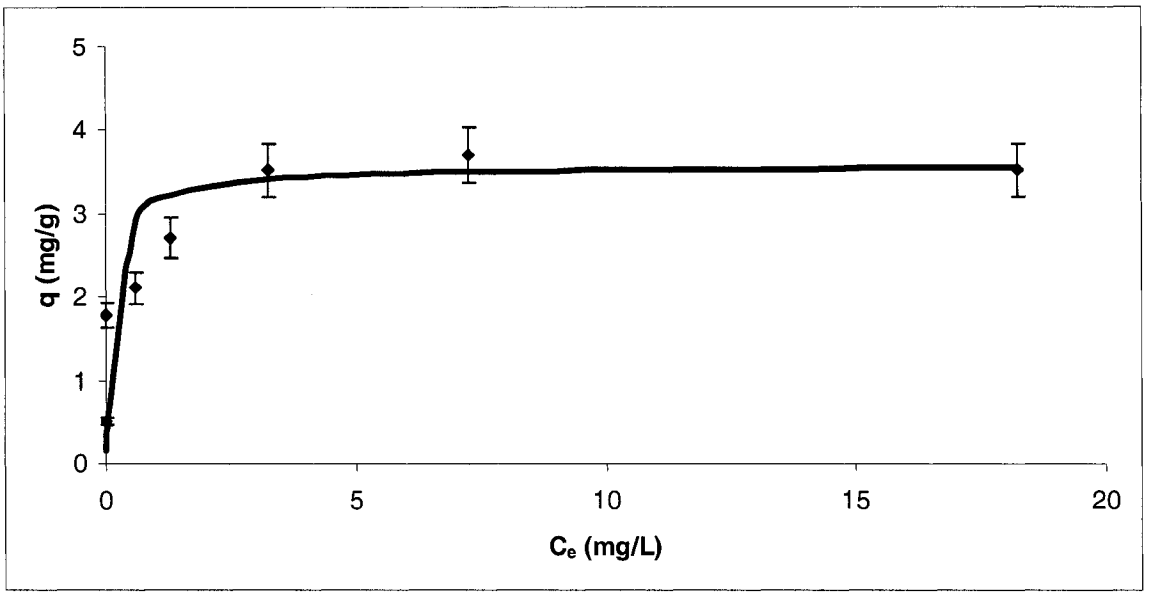

Figure 4-13. Theoretical Langmuir isotherm with experimental data points for non-viable biomass.

The maximum amount of Cr(VI) adsorbed per unit mass dry weight of non-viable biomass was also estimated using the Langmuir model. Non-viable biomass was found to have a q_{max} of 3.57 mg Cr(VI)/g dry weight.

A comparison between q_{max} for viable and non-viable biomasses is difficult since both differ in physical states. However, recalculating Cr(VI) sorption on the basis of dry weight for the viable biomass, its sorption capacity is about 88 mg Cr(VI)/g, since one gram of wet weight biomass corresponds to 0.06 g dry weight. Since 1 g of heat-dried biomass will have a volume of 4 mL when hydrated, it can absorb about 0.9 mg Cr(VI)/mL. Also, since 1 g of settled viable biomass has a volume of about 1.11 mL, then it can sorb about 4.74 mg Cr(VI)/mL. Table 4-3 summarizes the various calculated q_{max} values for viable and non-viable biomasses.

Table 4-3 q_{max} values for viable and non-viable biomass.

Sorption by viable biomass	Sorption by non-viable biomass
5.26 mg Cr(VI)/g wet weight	n/a
88 mg Cr(VI)/g dry weight	3.57 mg Cr(VI)/g dry weight
4.74 mg Cr(VI)/mL settled volume	0.9 mg Cr(VI)/mL hydrated volume

Although the q_{max} values for viable and non-viable biomasses are not strictly comparable, it is seen from the above table that the viable biomass performs better than the non-viable one regardless of the method used to express the sorption parameter. Others (Stasinakis et al. 2003) have studied the uptake of Cr(III) and Cr(VI) using activated sludge, and have determined a very low uptake capacity for Cr(VI) which did not exceed 15% of the added Cr(VI) for a studied range of 0.5 to 10 mg Cr/L. They also demonstrated the ability of activated sludge to remove all of the added Cr(III). Their study concluded that enhanced reduction of Cr(VI) to Cr(III) was necessary if the activated process was to be used as detoxification process for wastewater containing Cr(VI). They found an adsorption capacity of about 50 mg Cr(III)/g mixed liquor suspended solids MLSS (Stasinakis et al. 2003). Thus, the sorption capacity found for the cheese whey biomass for Cr(VI) of 88 mg/g dry weight biomass is even higher than that obtained for Cr(III) using activated sludge. These results confirm the rationale of this study for utilizing anaerobic sludge or biomass as an efficient biosorbent for Cr(VI).

4.1.5 Biomass Acclimation to Hexavalent Chromium

The results for acclimated phase I optimized biomass as per section 3.4.3.3 are given below. Figure 4-14 is a comparison of the CH_4/CO_2 ratio between acclimated and non-acclimated biomass at increasing Cr(VI) exposure levels.

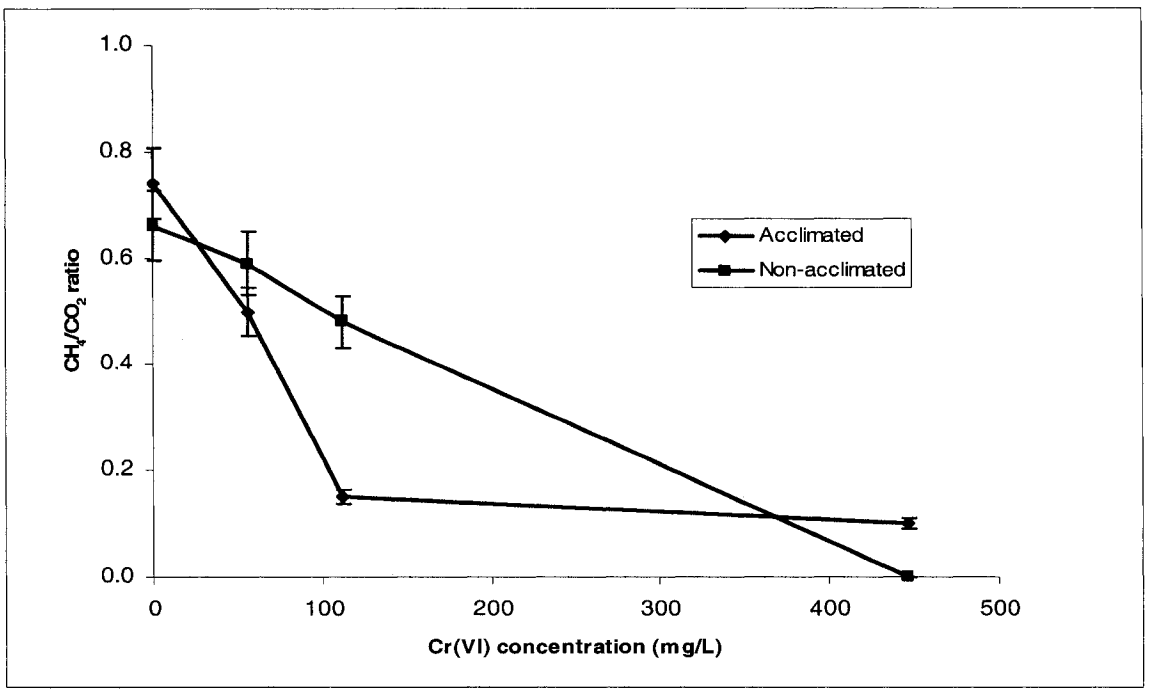

Figure 4-14. Comparison of biogas CH_4/CO_2 ratio for acclimated and non-acclimated phase I optimized biomass.

The purpose for acclimating the cheese whey biomass to Cr(VI) was to determine if the toxic effects of subsequent higher loadings of Cr(VI) could be diminished when compared to its non-acclimated counterpart. Studies have indicated that acclimation of activated sludge to heavy metals can mitigate the toxic effects of subsequent exposure (Yetis et al. 1999). It also been shown that anaerobic sludge acclimated to Cr(III) was more resistant to trivalent chromium toxicity than the non-acclimated one (Alkan et al. 1996). Although the initial

CH_4/CO_2 ratios for acclimated and non-acclimated biomass were similar in this study, the interval slopes of the ratios versus Cr(VI) exposure levels were different. The CH_4/CO_2 ratio of the acclimated biomass appears to fall off more sharply than that for the non-acclimated biomass indicating a greater relative decrease in methanogenesis. Although this phenomenon would also occur if CO_2 production was relatively increased, the actual CO_2 content of the biogas for each biomass was similar (Figure 4-15). The actual CH_4 content of the biogas for the acclimated biomass exhibited a sharp decrease at a Cr(VI) exposure level of 112 mg/L when compared that for the non-acclimated one (Figure 4-16). Thus, it would appear that the acclimated biomass was more sensitive to subsequent Cr(VI) loading. If the percent COD removal for both biomasses is examined (Figure 4-17), a sharp decline in COD removal for the non-acclimated biomass between the 112 and 446 mg Cr(VI)/L concentration interval can be seen. However, for the acclimated biomass a similarly sharp decline in COD removal is observed for the 56 to 112 mg Cr(VI)/L interval.

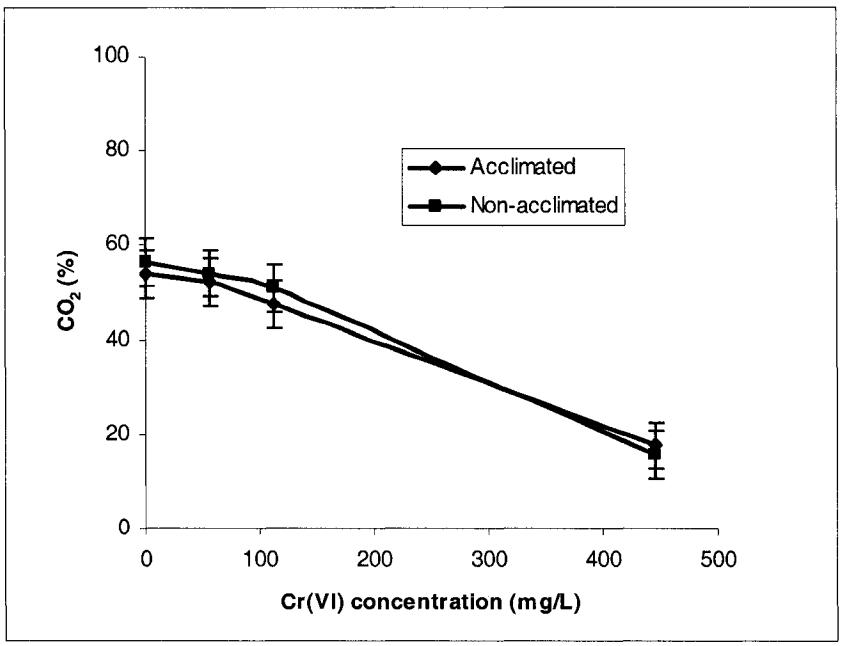

Figure 4-15 Biogas carbon dioxide content for acclimated and non-acclimated phase I optimized biomass.

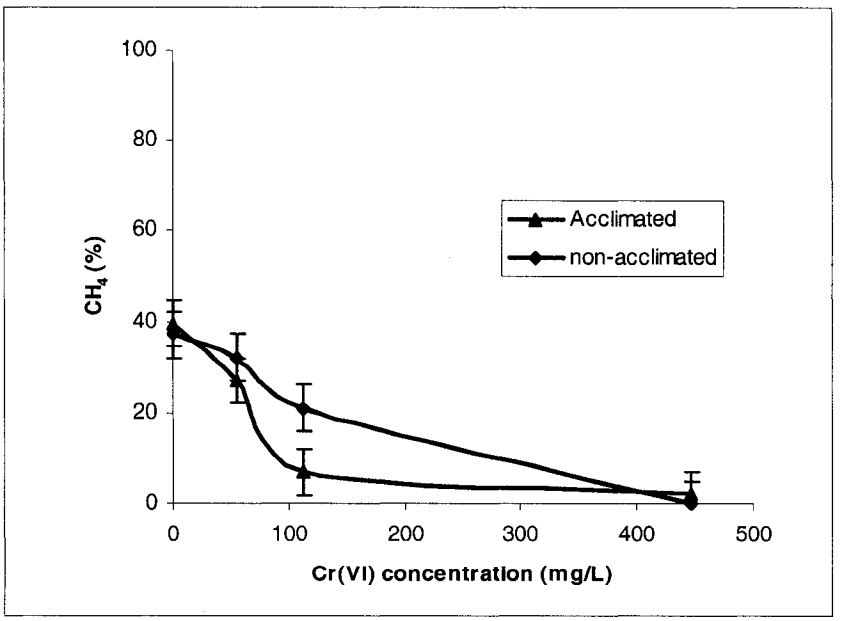

Figure 4-16 Biogas methane content for acclimated and non-acclimated phase I optimized biomass.

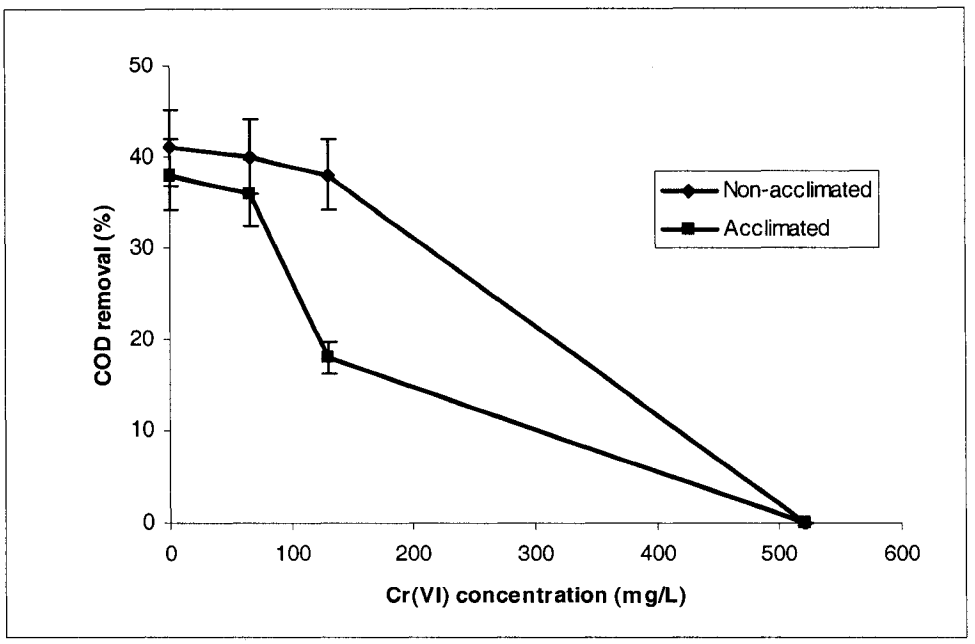

Figure 4-17. Comparison of soluble COD removal between acclimated and non-acclimated phase I optimized biomass. BARS

However, if the total amount of Cr(VI) added during the acclimation period is calculated, and it is assumed that 100% of the chromium is sorbed, an estimated, accumulated loading of 60 mg Cr(VI)/L settled biomass can be obtained. Thus, the actual dose of Cr(VI) at the 112 mg/L exposure level would have been 135 mg Cr/L settled acclimated biomass compared to 75 mg Cr/L for the settled non-acclimated biomass. Figure 4-18 gives the percent COD removal versus dose taking into account the estimated 60 mg/L settled biomass already reached by the acclimated biomass. It can be observed that the sharp decline in COD removal efficiency now occurs within the same interval indicating that the reduction in CH_4/CO_2 ratio as well as COD removal efficiency is due to the toxic effect of Cr(VI) and not to any differences between acclimated and non-acclimated biomasses. In retrospect, this final accumulated level may have been too high. However, a little difference between the control biomass (no chromium

initially added) for both acclimated and non-acclimated biomass can be seen with respect to CH_4/CO_2 ratio and COD removal efficiency; in fact the corresponding values for the acclimated biomass are slightly less than those obtained for the acclimated system. Since there appears to be no benefit in using acclimated biomass based on the control values, the decision was made to use non-acclimated, phase I optimized biomass in reactor one of the ASBR experiment. However, an initial four day period for gradually increasing Cr(VI) loading protocol was used to minimize any shock loading effects (see section 3.4.4).

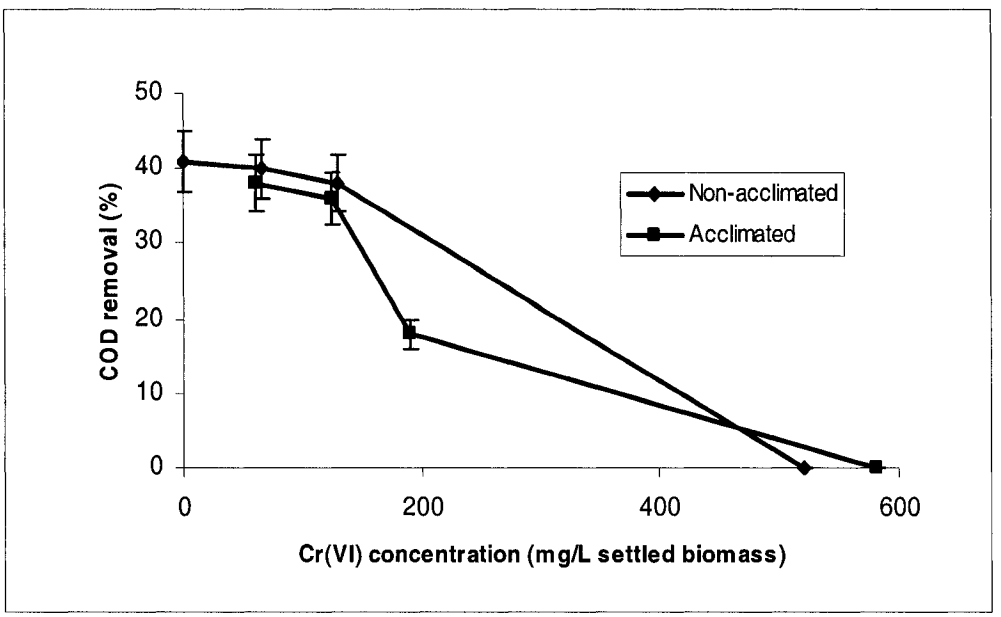

Figure 4-18 Comparison of soluble COD removal between acclimated and non-acclimated phase I optimized biomass taking into account the accumulation of Cr(VI) during the acclimation period.

4.1.6 Hexavalent chromium Removal by Viable and Metabolically Inhibited Biomass

In order to determine if the removal involves a biologically mediated phenomenon or a simple physico-chemical one, a comparison was made between active, viable biomass and biomass inactivated by a metabolic inhibitor. Using fermentation tubes it can be easily screened for the inhibition of biogas production.

Figures 4-19 and 4-20 are photographs of the actual fermentation tube experiment. Control tube (viable biomass) shows gas production. Some of the biomass granules have actually been pushed up by the biogas and can be seen sticking on the top of the side arm chimney. The fermentation tube having even the lowest concentration of azide used (0.25 mL of 5% sodium azide) showed no biogas production.

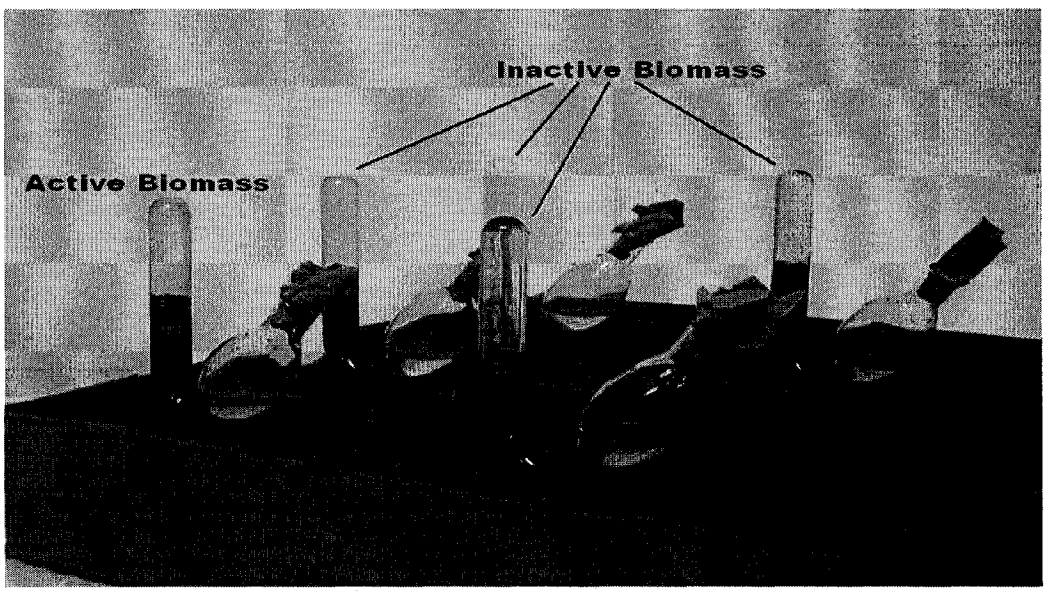

Figure 4-19. Inhibition experiment at time zero.

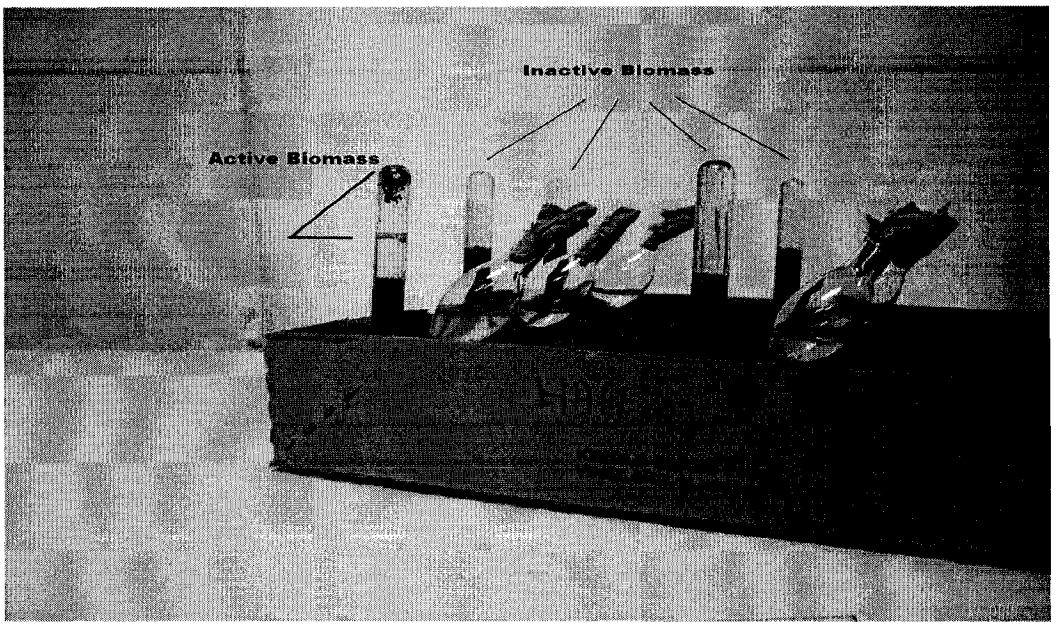

Figure 4-20. Inhibition experiment after 24 h.

Results of the subsequent sorption test for Cr(VI) using viable and metabolically inhibited biomass (Figure 4-21) shows that as much as 25% (or more) of the removal of Cr(VI) involves a metabolically mediated process. Others have shown using actinomycetes that reduction of Cr(VI) was decreased by 50% when azide was added to a culture even though all of the reduction of Cr(VI) was mediated by active metabolic processes (Laxman and More 2002). Thus, it is possible that metabolically mediated removal of Cr(VI) from the liquid phase might have played a larger role than the results of this experiment indicated. Metabolic processes could involve Cr(VI) redox mediated reduction to Cr(III) in the extra-cellular soluble fraction, which would presumably result in the precipitation of Cr(III) as the insoluble hydroxide, and/or active uptake of Cr(VI) followed by intra-cellular reduction (Laxman and More 2002, Gadd 1990, Priester et al. 2006). Differentiating between these two would require measuring chromium in both

extra-cellular and intra-cellular compartments in the biomass. Since a biomass granule is a complex system comprised of bacterial cells and large amounts of extra-cellular material, determining what levels of chromium occurred in the cytoplasm as opposed to what was bound to the external cell membranes and extra-cellular material was beyond the scope of this study. Bioaccumulation as opposed to biosorption can at best be inferred by comparing active and metabolically inactive biomass.

No Cr(III) was detected in the supernatant or liquid phase for either active or metabolically inhibited biomass. If the metabolically inhibited biomass is considered, the removal of Cr(VI) most likely involves sorption to the biomass granules followed by physico-chemical reduction, whereupon the Cr(III) is either complexed with the organic material of the granules or precipitated onto the surface as insoluble hydroxide. If the uninhibited biomass is considered, the absence of Cr(III) in the liquid phase, in addition to the removal scenario presented for the inhibited biomass, may involve cellular uptake of Cr(VI) with subsequent reduction to Cr(III). It is also possible that some extra-cellular Cr(III) resulting from the biosorption of chromate could also be taken up actively by viable cells. Trivalent chromium toxicity in anaerobic digestion processes was found in many cases to be a result of intracellular accumulation of chromium (Alkan et al. 1996). In conclusion, the results indicate that metabolic processes may play an important role in the removal and reduction of Cr(VI). Reduction and removal by physico-chemical processes cannot be overlooked; however, it

seems that regardless of the mode of the reduction and removal, essentially all the chromium is sequestered on or in the biomass granule compartment.

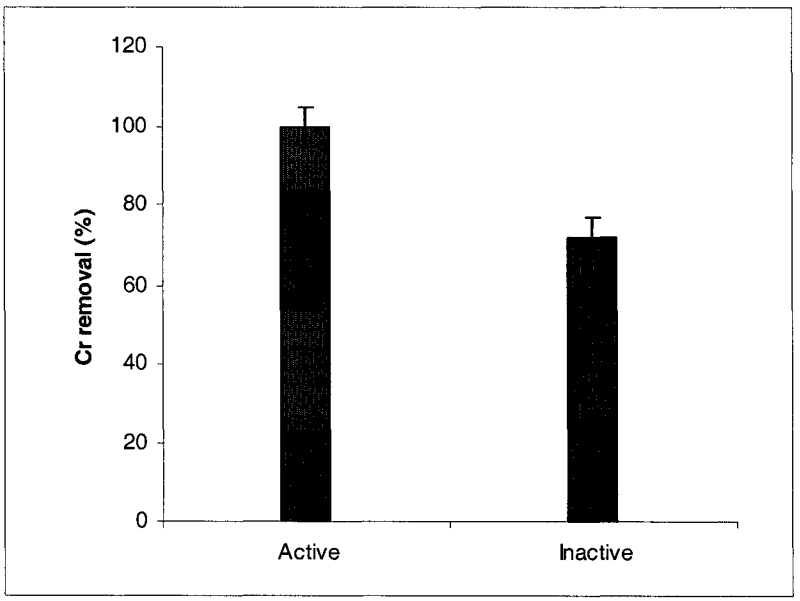

Figure 4-21. Removal of Cr(VI) using viable and metabolically inhibited biomass.

4.2 Two-phase ASBR Experiment

The goal of this experiment was to evaluate the removal of hexavalent chromium from wastewater using living anaerobic biomass without adversely affecting the anaerobic digestion process in terms of methane production. In reactor one the removal of Cr(VI), as well as the conversion of organic compounds to VFAs occurred. Biomass in reactor two, receiving the VFAs from reactor one, free of any traces of chromium that can lead to process failure, could maintain optimal methane production.

Results from the ASBR experiment are presented below. Figure 4-22 shows chromium and soluble COD removal attained by reactor one. The data clearly show the removal efficacy for Cr(VI) by the cheese whey biomass. The experiment was concluded when chromium levels in the effluent were greater than 1 mg/L. A drop in COD removal reached a level of about 60% of the initial COD removal by day 28, when biomass accumulated about 613 mg Cr(VI)/L settled biomass (based on an estimated biomass volume of about one liter); which represents over 99% of added Cr(VI).

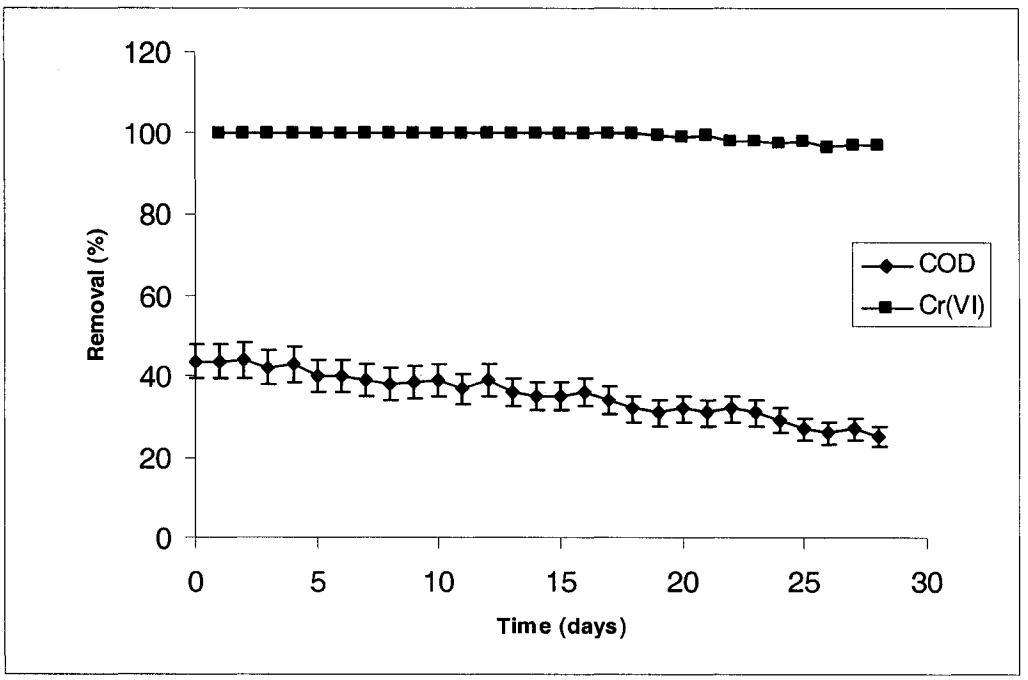

Figure 4-22. Percent of COD and chromium removal in reactor one over time.

This gradual drop in COD removal is most likely due to the effect of chromium on the more sensitive methanogens as the next figure implies (Figure 4-23), for a dramatic decrease in the CH_4/CO_2 ratio of the biogas over time can be seen. If a calculation of the accumulation of chromium in reactor one for day 28 was made,

a total loading of chromium of 613 mg can be obtained (see section 4.4). Referring back to the batch tests, an ID50 of about 283 mg Cr(VI)/L settled biomass was determined based on COD removal for phase I optimized biomass. Since, as was previously mentioned, the maximum reduction in COD removal for the ASBR experiment was about 40%, at an accumulated chromium level of about 613 mg Cr(VI) in reactor one which contained approximately 1 liter of settled biomass (see section 4.3), the ASBR system (semi-batch experiment) exhibited a greater resistance to Cr(VI) than that for the batch test. An ID40 of about 270 mg Cr(VI)/L settled biomass can be calculated for the batch test data (Figure 4-6). Thus, an ASBR system exhibits an increase in resistance to Cr(VI) loading with respect to COD removal when compared to the batch test experiment.

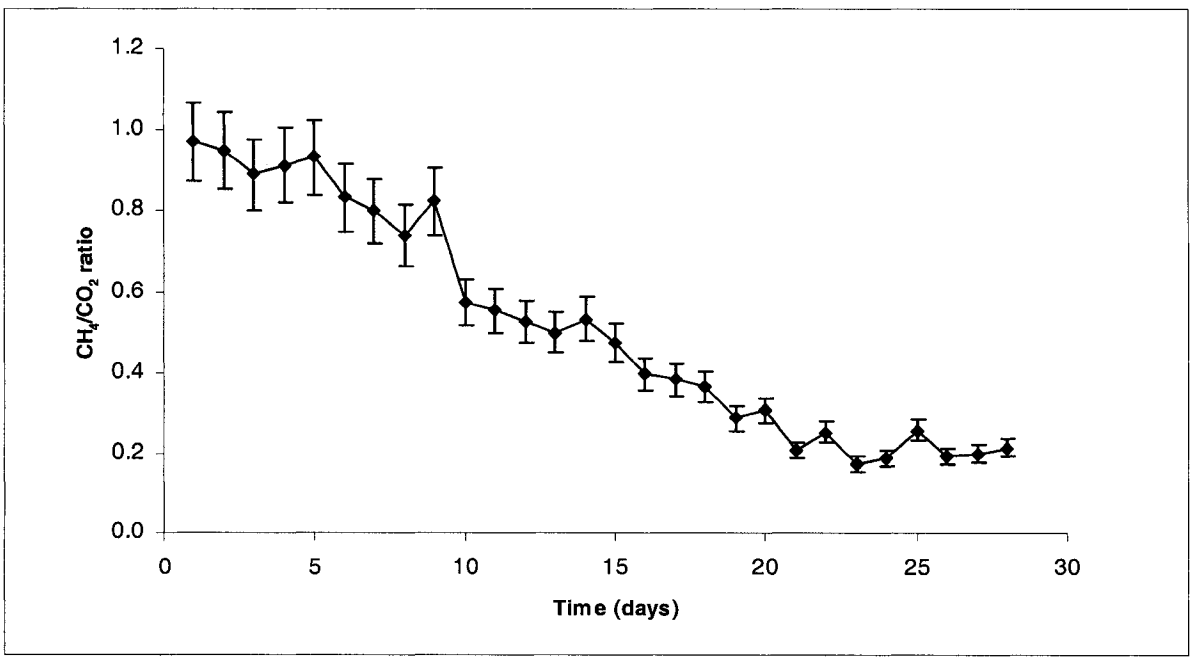

Figure 4-23. Change in CH_4/CO_2 ratio in reactor one over time.

Figure 4-24 gives the actual percentage of CH_4 in the produced biogas based on biogas production volumes and the void volume (2.0 liters) of the reactor headspace and gas collection system. A decrease in the methane content over time can be observed.

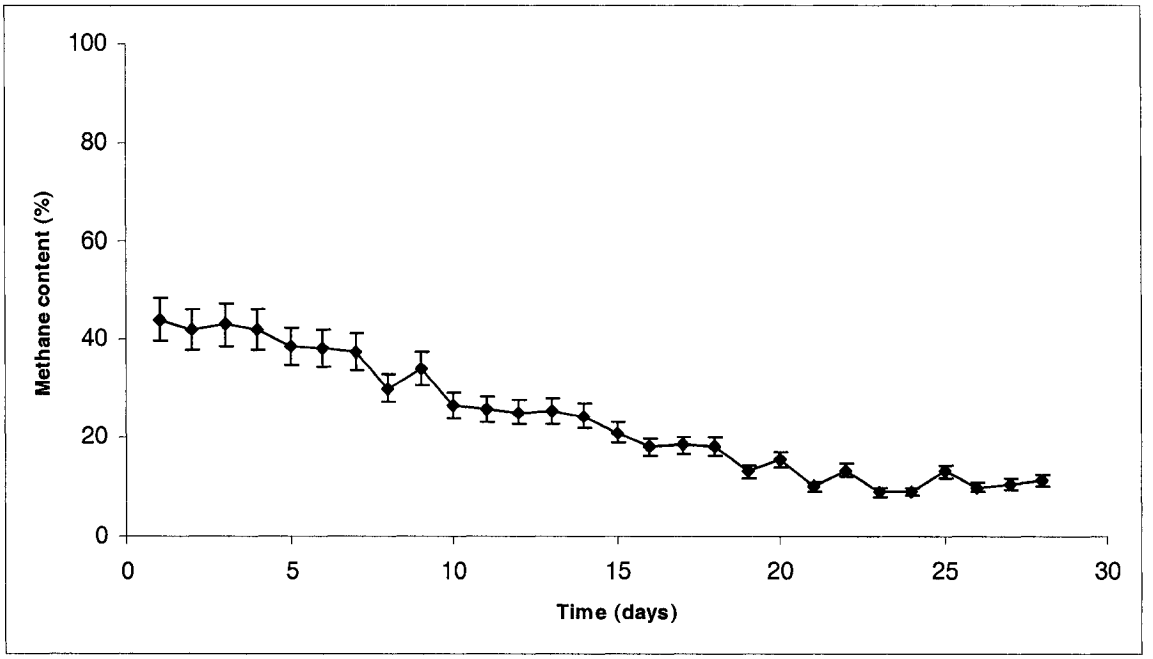

Figure 4-24. Methane content in the biogas produced by reactor one.

Figure 4-25 provides the daily volumes of biogas produced by reactor one. A slight decrease over time can be observed which amounts to about 20%. This overall decrease is certainly due to a reduction in methanogenic activity in reactor one. It has been observed by others that a loss in biogas production was accompanied by a reduction in methane production, due to toxic effects of Cr(III) and an increase in CO_2 production (Alkan et al. 1996). An increase in CO_2 production of about 15% was observed. This phenomenon tends to reduce the CH_4/CO_2 ratio more than might be expected.

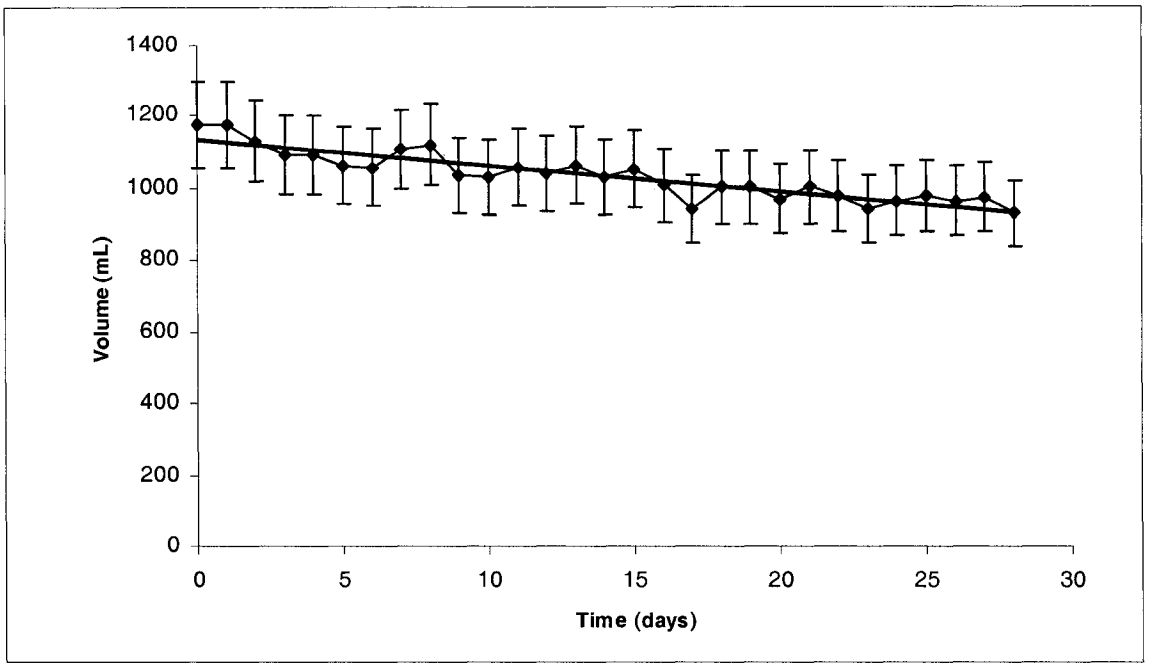

Figure 4-25. Daily volumes of biogas produced by reactor one.

Based on percent methane content and daily volumes of biogas, the change in daily production of methane with time can be estimated (Figure 4-26).

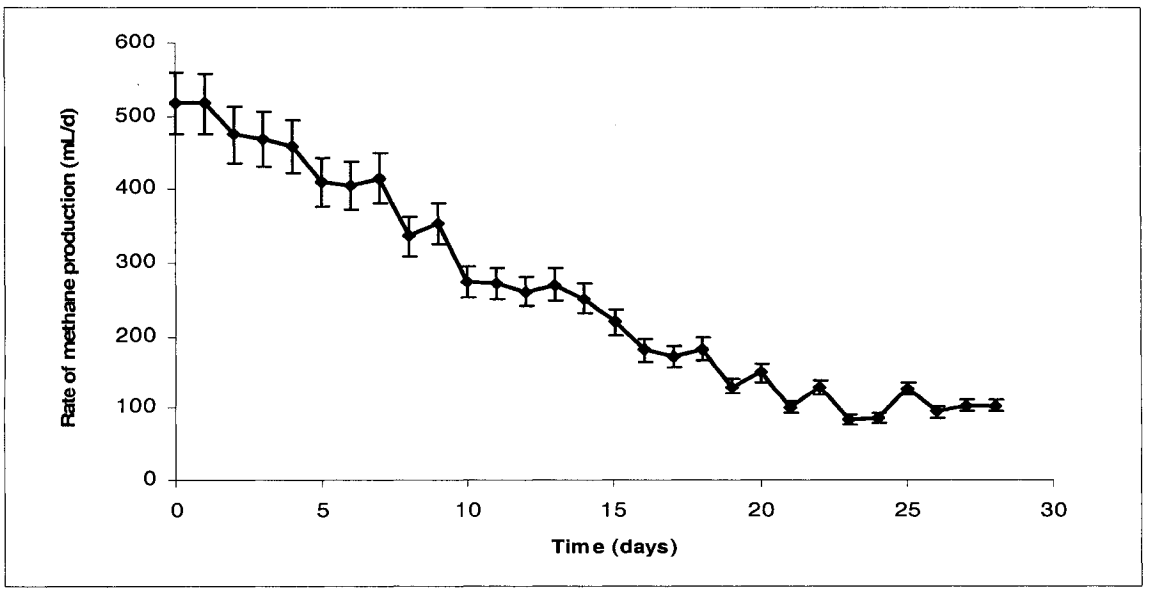

Figure 4-26. Change in daily methane production over time in reactor one.

Figure 4-27 shows the effluent VFA profile for reactor one. The daily average VFA concentration in the effluent of reactor one was about 2200 mg/L. A maximum decrease in VFA production of about 25% was observed by day 28 when compared to day 0.

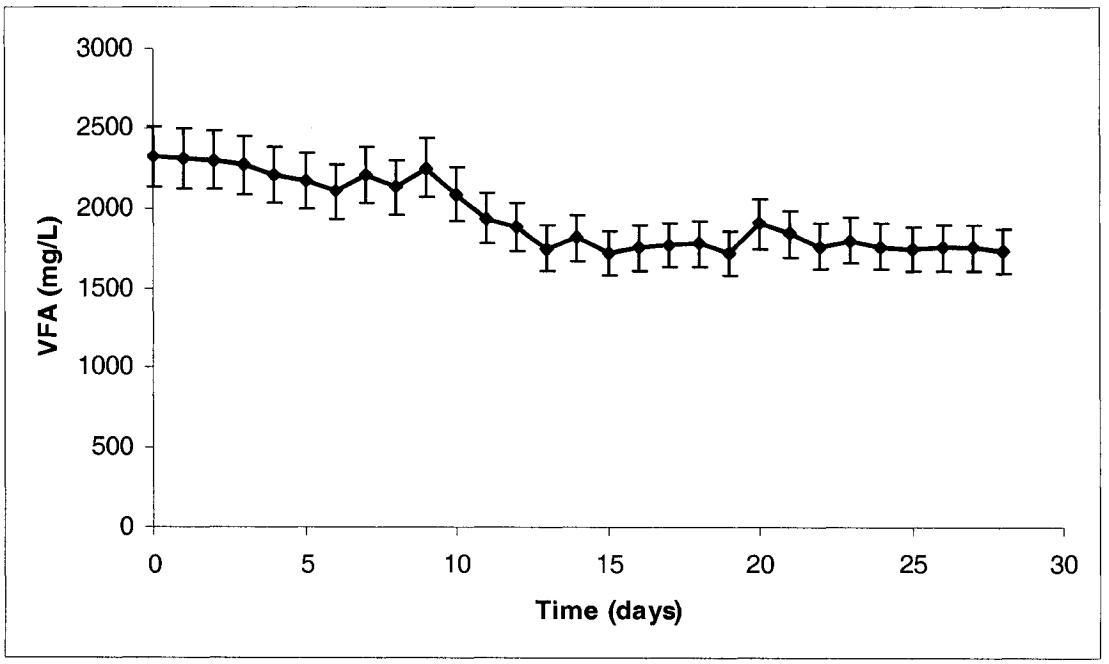

Figure 4-27. VFA concentration in the effluent of reactor one.

Since the VFAs are formed by acidogens and removed by methanogens, the actual production of VFAs might be expected to increase as methane production in reactor one falls off. Methane production up until day 4 in reactor one remained close to the production level for day zero when no Cr(VI) was added to the system; however, after day four a steady decline in methane production was observed. Yet a reduction in the VFA concentration in the effluent of reactor one is observed during the declining phase of methane production. This is believed to be due to the inhibitory effect of VFAs on methane production. As methane

production decreased, the removal rate for VFAs in reactor one also decreased; possibly resulting in a decrease in VFA production. In addition, the accumulated chromium in reactor one eventually will have a detrimental effect on acidogenic activity, which might also result in reduced VFA production.

Since Cr(VI) was slowly introduced into reactor one in the ASBR experiment, as opposed to short term exposure (2 days) of large concentrations of Cr(VI) it can be reasonably assumed that Cr(VI) reduction to Cr(III) would be more likely to take place given the extended duration of the test. Cr(III) is known to be less toxic to the biota in general. This may account for the increased resistance to Cr(VI) exhibited in the ASBR system to a reduction in COD removal. The ASBR experiment was concluded not because chromium had an adverse effect on acidogenic activity since VFA production was maintained at a fairly high level throughout the duration of the experiment, but because Cr(III) was found in the supernatant, which was the influent for reactor two where methanogenic activity was maintained. It must be stressed that no chromium in the form of Cr(VI) was detected in the supernatant of reactor one. Intermittent oxidation-reduction potential measurements carried out for reactor one indicated a highly reducing environment. The average ORP value for reactor one was -20 ±10 mV.

Figure 4-28 shows the percent removal of soluble COD and VFA for reactor two. The average soluble COD removal was maintained at about 94% for the duration

of the test. The VFA removal profile for reactor two shows almost complete removal or consumption of VFAs. The average VFA removal was about 97%.

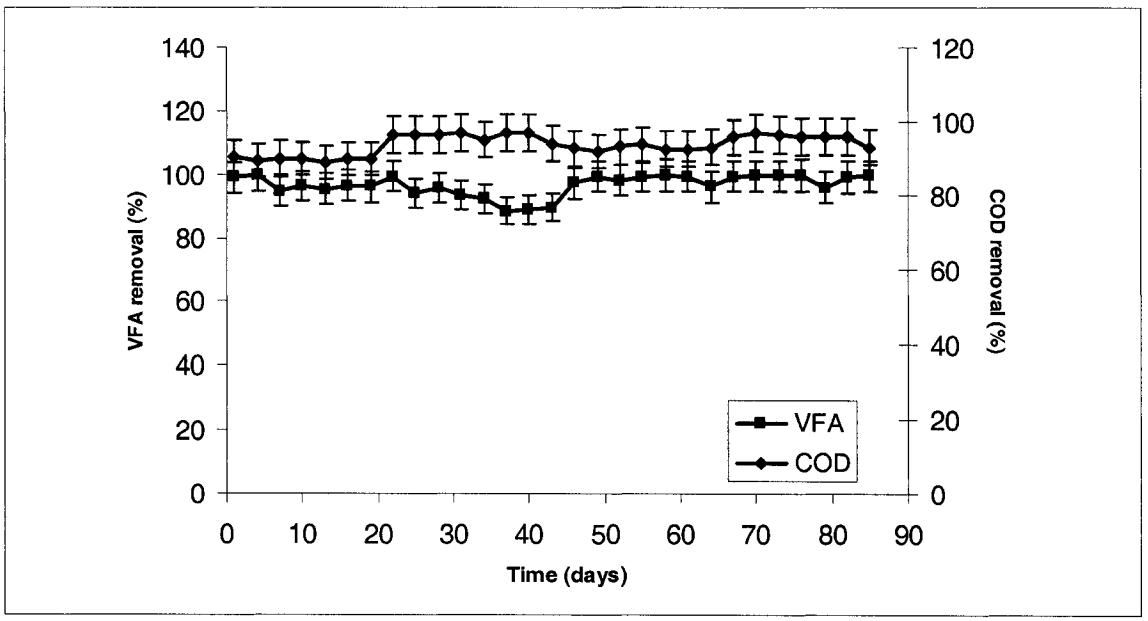

Figure 4-28. Percent of soluble COD and VFA removal in reactor two.

Figure 4-29 shows a typical reduction in soluble COD in reactor two over the three day retention time. The results represent an average based on measurement taken at days 1, 40 and 82. The overall average reduction in COD was about 2.87g COD/L for the one liter working liquid volume. This represents a COD removal of about 97%. The average total biogas production for a three day detention time was about 1.5 liters.

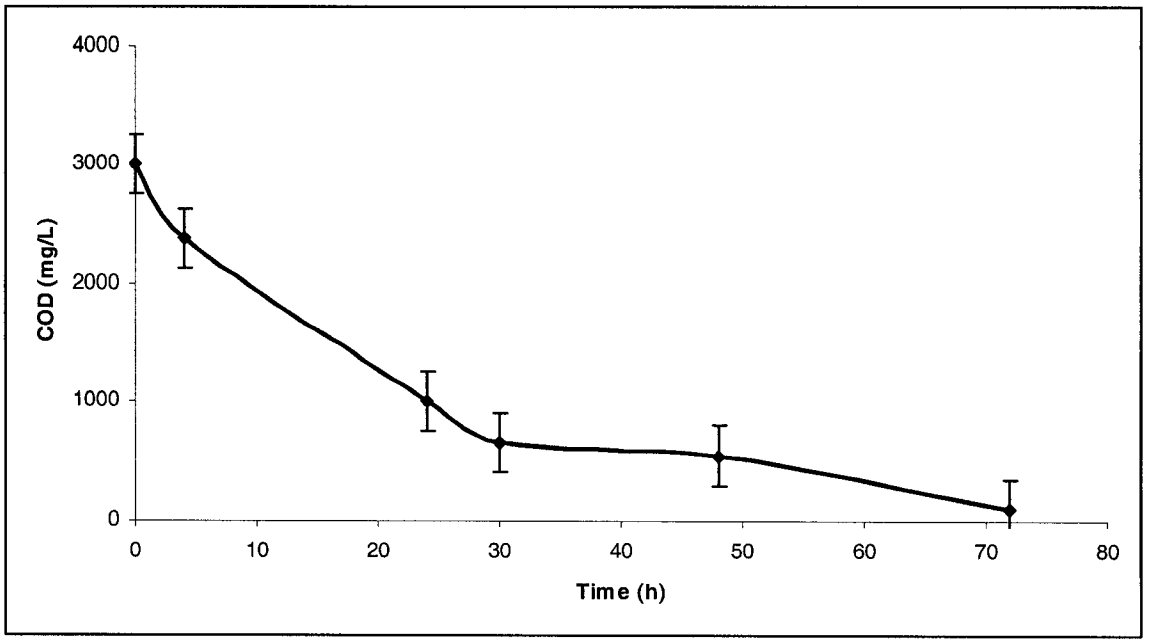

Figure 4-29. Average soluble COD in reactor two during the 3 day retention time.

The methane content of this biogas was 82%. Thus the methane volume of the biogas was about 1.23 Liters. Therefore, about 0.43 liters of methane produced per gram of soluble COD removed. This value is close to the theoretical methane production versus COD consumption for an anaerobic reactor. A value of about 0.39 L CH_4/g COD at 35 °C is typical (Speece 1996). Although typical biogas methane content in anaerobic digesters is between 60 and 70%, the high methane content in the biogas of rector two can most likely be attributed to the suitability of the feedstock which is partially metabolized soluble sucrose with a high VFA content. It should be noted that others have also achieved methane contents greater than 80% in the biogas of their anaerobic digesters (Parawira et al. 2004, Natural Resources Canada 1997).

The results of the ASBR experiment demonstrate the efficacy of the two- phase ASBR for the treatment of hexavalent chromium-containing effluents. Phase I microbial activity in the cheese whey biomass has proven to be fairly tolerant to Cr(VI). Results indicate that phase II methanogenic activity in reactor one is gradually reduced with increased Cr(VI) loading. VFA production was satisfactorily maintained in reactor one, and the effluent of reactor one, which is the feedstock for reactor two, remained essentially free of chromium until levels greater than 600 mg Cr(VI)/L settled biomass in reactor one were reached; thus, maintaining optimum methane production in reactor two. It is interesting to note that even though methanogenic activity was reduced by about 75% in reactor one by the end of the experiment, a reduction in VFA production of only 25% was observed. Thus, if at least 25% methanogenic activity is maintained under Cr(VI) loading, VFA production will apparently be maintained at a fairly high level.

4.3 Biomass Characterization

The biomass in each reactor of the ASBR experiment was subjected to solids characterization at the beginning and end of their operational period; which amounted to 42 days for reactor one and 82 days for reactor two. This characterization is given in Table 4-4 and represents the average of two replicate runs for each reactor.

Table 4-4. Biomass characteristics before and after the ASBR experiment.

Parameter	Initial reactor one Biomass (mg/L)	Initial reactor two Biomass (mg/L)	Reactor one Biomass (mg/L)	Reactor two Biomass (mg/L)
Total solids (TS)	34,920	34,420	34,200	32,020
Total volatile solids (TVS)	28,970	28,490	27,420	26,500
Total fixed solids (TFS)	5,950	5,930	6,780	5,520
Total suspended solids (TSS)	710	690	820	760
Total volatile suspended solids (TVSS)	690	670	780	735
Total fixed suspended solids (TFSS)	20	20	40	25
Settled volume (mL/L)	900	860	810	750

Average errors for duplicate analysis were: TS (±13%), TVS (±16%), TSS (±33%), TVSS (±49%), and settleable volume (±15%). TFS and TFSS were calculated values.

The initial average settled biomass volume for phase I optimized biomass was about 900 mL/L based on the Imhoff cone settling test. For the phase II, the optimized biomass in reactor two was about 860 mL/L. After each reactor was taken off line, there appeared to be a reduction in the average settled biomass volume for each reactor. This reduction amounted to about 10% for reactor one and 13% for reactor two. Although the experimental error involved in this determination is fairly large due to the gradation scale of the measuring cone, a reduction in biomass volume for both reactors is possible. Mixing biomass granules too vigorously can reduce their size due to shearing forces which can remove material from the granule surface. This coupled with the low biomass yield for anaerobic reactors in general could result in a net reduction in granular size.

Based on the average TVS, an estimate of the volatile solids (mg) in each reactor at the beginning and end of their run can be obtained. An overall decrease in volatile solids of almost 15% for reactor one and 19% for reactor two can be estimated. The continuous removal of COD during the operational period for reactor one indicated ongoing metabolic activity, albeit at increasingly lower rates as the experiment progressed, but the estimated 15% reduction in volatile solids indicates a net loss in biomass over the 42 day run time. Reactor two ran optimally for 82 days, so again the reduction seems to be due to a net loss in biomass. The net loss of biomass could only presumably occur by two routes: endogenous metabolism of the biomass or loss of VSS during the withdraw phase. Although total gas production was not measured on a daily basis for reactor two, it was for reactor one (Figure 4-25). An increase in endogenous metabolic activity should result in an increase in biogas production when compared to initial values; however, relative biogas production decreased over time. Since a relative increase in VSS in the liquid phases of reactors one and two was observed, it is more likely that the reduction in TVS is due to the removal of suspended material during the withdraw phase.

This loss however is of concern mainly with respect to reactor one where loss of suspended solids contaminated with chromium could carry over to reactor two. For anaerobic digestion of carbohydrates (the carbohydrate sucrose was the original substrate for reactor one) the biomass yield is typically 0.024 mg biomass/mg BOD_5 consumed (Tchobanoglous and Burton 1991). Based on an

estimate of about 3000 mg COD consumed for reactor one per day, an overall increase in biomass of about 72 mg biomass/day for reactor one can be roughly estimated since in the case of the sucrose substrate, which is completely biodegradable, BOD and COD would be similar. Using the difference in initial and final volatile solids for the biomass in reactor one, an estimated daily loss of about 92 mg/day can be calculated. This coupled with the above estimated biomass yield of 72 mg biomass/day for reactor one yields a total loss of about 164 mg biomass/day. Although not listed in Table 4-4, a TVSS analysis of five random samples of the effluent from the withdraw cycle of reactor one yielded an average TVSS of 270 ± 165 mg/L, or a range 105 to 435 mg TVSS/L.

Thus, considering the overall low precision of the TVSS measurement and the use of published biomass yields for anaerobic reactors, the reduction in TVS could be accounted for through loss of TVSS during the withdrawal phase for reactor one. Carry over of chromium seems to be of little concern during the first half of the run time for reactor one as the ASBR experiment demonstrated; however, carry over will be a concern once accumulation of this metal has increased. The results of the solids analysis again indicate the need to investigate a more gentle mixing regime, for reactor one (only a continuous stir option at the lowest speed of 50 rpm was available for the *"New Brunswick Scientific* Model *Bioflo 110 Fermentor/Bioreactor*" used in this study).

The TVSS in Table 4-4 represents that for the liquid fraction remaining after the withdraw phase for each reactor. The average error was not included in the table for the measurements, but for reactor one, after the 42 day run period, the actual value was 690 ± 197 mg TVSS/L, or a range of 493 to 887 mg TVSS/L. Thus, taking the rather large errors into account, the lower limit of this TVSS fraction would seem to be comparable to the upper limit for the TVSS of the withdrawn effluent (105 to 435 mg TVSS/L). This greater TVSS value may result from the removal and pouring of the biomass into the Imhoff cone or may represent an actual difference in the TVSS in the liquid fraction of the upper and lower sections of the reactor. In other words, there is a higher level of TVSS in the liquid proximate to the biomass granules as opposed to that overlying the biomass bed. Why this difference was observed is not clear. One further point which deserves mention is in regards to the total fixed solids (TFS), which estimates the inorganic fraction of the settled biomass granules.

The difference in TFS for reactor one at the beginning and end of the experiment amounted to an average of 830 mg. It was determined by acid digestion (section 4.8) that the spent biomass from reactor one had accumulated about 1140 mg chromium/L settled biomass; which would amount to 923 mg chromium, based on a final biomass settled volume of 810 mL. Thus, given the experimental error involved, the increase in TFS for reactor one is most likely due to sorbed chromium. It appears that the mass balance for chromium in reactor one can be accounted for in an increase in TFS. Photographs of the Imhoff cone test (for

final reactor one mixed biomass) and the liquid effluents from reactors one and two are shown below (Figures 4-30 and 4-31).

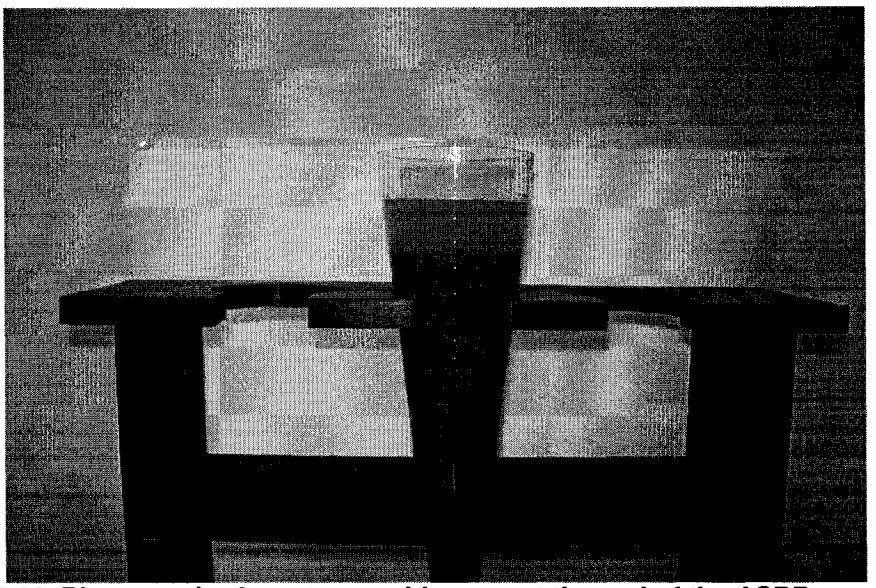

Figure 4-30. Photograph of reactor one biomass at the end of the ASBR experiment.

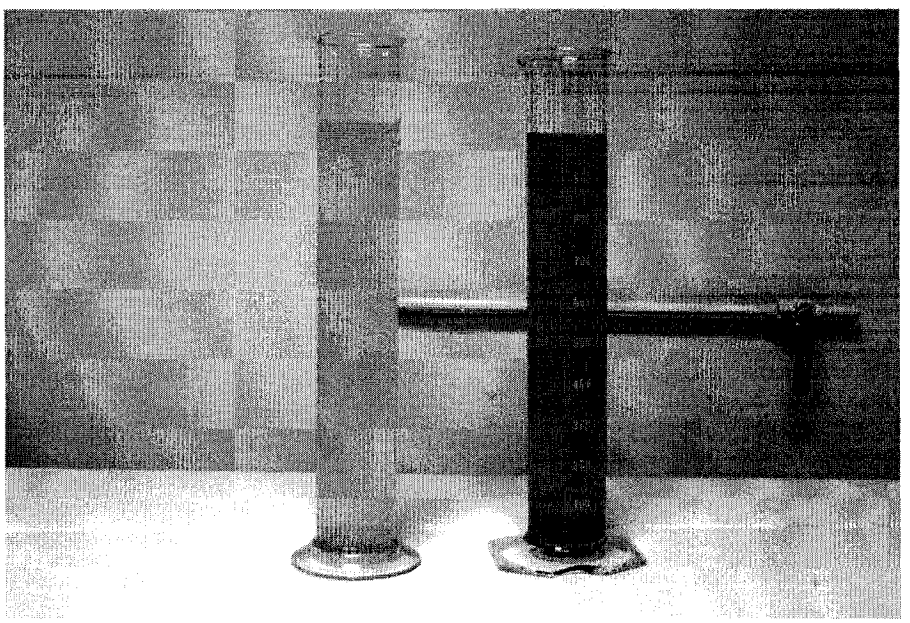

Figure 4-31. Photograph of the effluents from reactor one and two.

4.4 Volume of Wastewater Treated

At the end of the ASBR experiment, the volume of treated simulated wastewater was calculated based on the quantity of one-liter volumes that were processed. Subsequent to the addition of 4 volumes of 0.1, 1, 5 and 12.5 mg/L Cr(VI) each, 24 one-liter volumes of simulated wastewater, containing 25 mg/L of Cr(VI) were treated until chromium levels in the liquid phase were greater than 1 mg/L in reactor one. Thus, about 25 one-liter volumes of treated wastewater can be estimated based on the above additions. Figure 4-32 gives the average Cr(III)-concentration in the effluent from reactor one for the duplicate runs. No Cr(VI) was detected. The minimum detection limit for the diphenylcarbazide spectrophotometric method for chromium determination is about 0.01 mg/L (EPA 2003).

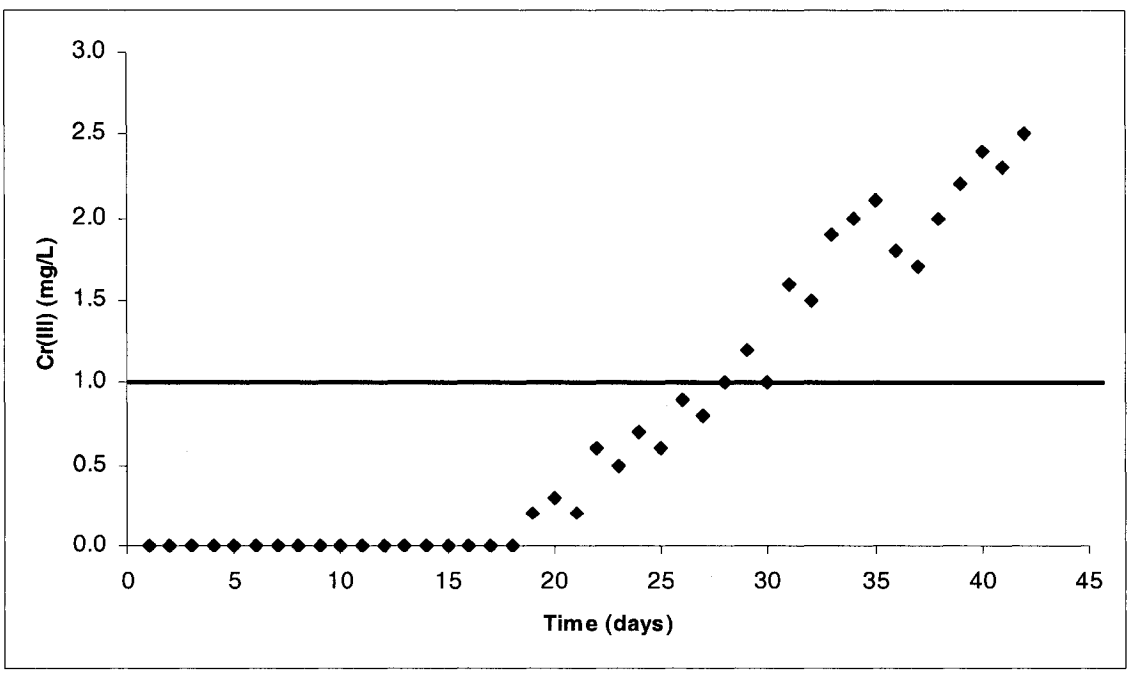

Figure 4-32. Cr(III) concentrations in reactor one effluent.

The total mass of hexavalent chromium that was removed by the biomass granules can be estimated based on the four initial lower one-liter Cr(VI) additions which mounted to a total input of 18.6 mg Cr(VI) and the 24 one-liter addition cycles of 25 mg Cr(VI)/L, as well as taking into account chromium found in the supernatant, which amounted to an accumulated total of 5.8 mg/L up to and including day 28. The overall estimated removal is 613 mg Cr(VI).

Since 613 mg of chromium had accumulated in reactor one, a maximum Cr(VI) removal per unit settled volume of biomass can be estimated at about 681 mg Cr(VI)/L settled biomass, based on the initial settled biomass volume of 900 mL. If the calculation is based on the final biomass volume for reactor one, which was 810 mL, a value of 757 mg Cr(VI)/L settled biomass can be estimated. Although the experiment was terminated with respect to addition of reactor one effluent to reactor two, when chromium levels in the liquid phase of reactor one were detected at levels greater than 1 mg/L, one-liter cycle additions of Cr(VI) to reactor one were continued for an extended two week period. During this time concentrations of chromium in the liquid phase remained between 1.1 and 2.5 mg/L. Thus, by the end of the experiment a total accumulation or chromium removal of about 950 mg can be calculated. Based on an initial settled volume of biomass this amounts to a sorption level of about 1.06 g Cr/L settled biomass. Sorption experiments had found a q_{max} of about 4.7 g Cr(VI)/L settled biomass (Table 4-3). The biomass in reactor one could thus have removed much more Cr(VI) based on its q_{max} value but the goal of this experiment was to completely

exclude chromium from reactor two. Figure 4-33 shows the relative percent reduction with respect to initial, or day zero (no chromium added), for COD removal, methane production, and VFA content in the effluent for reactor one. With respect to VFA in reactor one, a production rate based on added sucrose substrate is difficult to determine since residual liquid phase after each withdraw cycle will contain VFAs. The day zero residual liquid phase will also contain VFAs since it was maintained for thirty days with the same substrate, and fill and withdraw cycles as the chromium addition cycle regime. In addition, VFAs are simultaneously removed due to conversion to methane. However, the daily content of VFAs in the liquid phase at the withdrawal cycle can be thought of as accumulating based on net production. Thus, if the daily VFA content of the effluent is compared to the initial VFA content at day zero, a rough comparison of relative net production can be inferred.

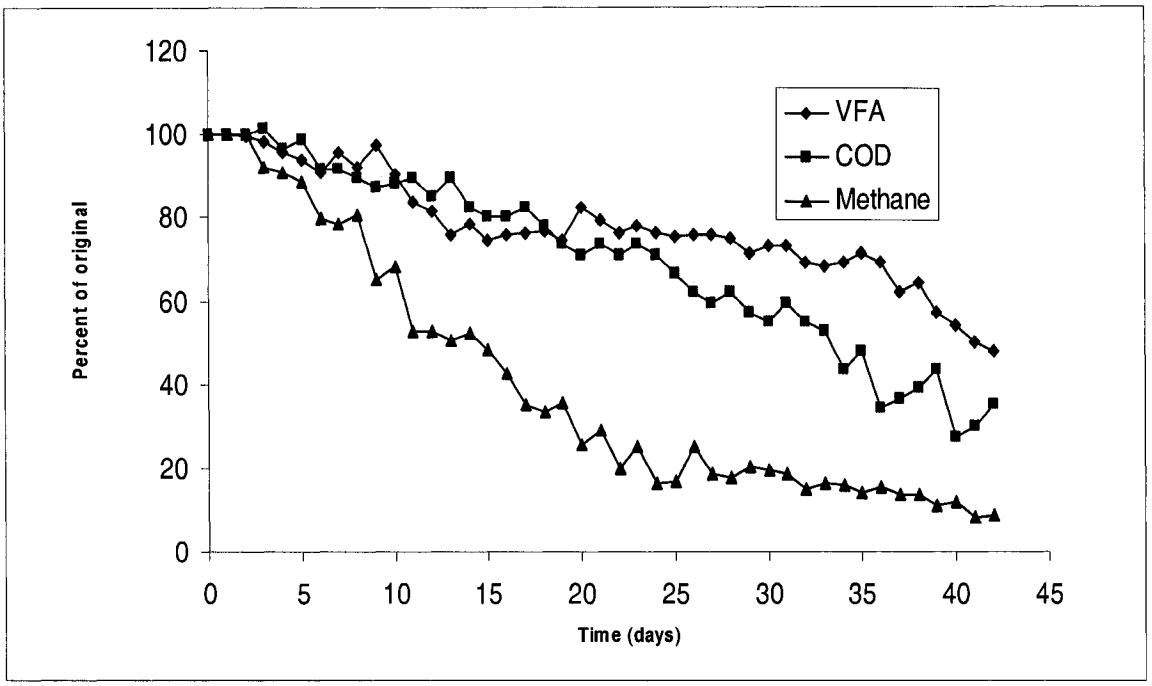

Figure 4-33. Relative percent reduction of COD removal, VFA concentration, and methane production for reactor one over time.

Figure 4-33 shows a decline in these parameters, relative to the day zero control value. By day 42, methane production was reduced by 91%, COD removal was reduced by 65%, and net VFA production was reduced by 52%.

4.5 Cumulative Hexavalent Chromium Toxicity to Cheese Whey Biomass in the ASBR Experiment

The batch test was used as a range finding tool to probe the toxicity of Cr(VI) to the cheese whey biomass using the removal of COD as the effect parameter. It should be recalled that for practical purposes, and based on the results of adsorption tests, to use a "dose" as opposed to a concentration of Cr(VI) that affects 50% of the test population, as the independent variable. The effect, or the inhibition of COD removal, compared to the control gave a 50% inhibition "dose"

or an ID50 of 283 mg Cr(VI)/L settled biomass. This toxicity estimate is based on single additions of fairly high Cr(VI) doses which is typical of short term, acute bioassays. The ASBR experiment is similar to a long term, chronic bioassay. In this case the effect parameter is inhibition of some measurable metabolic byproduct or process versus an accumulated dose. Figure 4-34 shows the relative percent reduction when compared to control values for COD removal, methane production, and net VFA accumulation versus accumulated chromium per liter settled biomass.

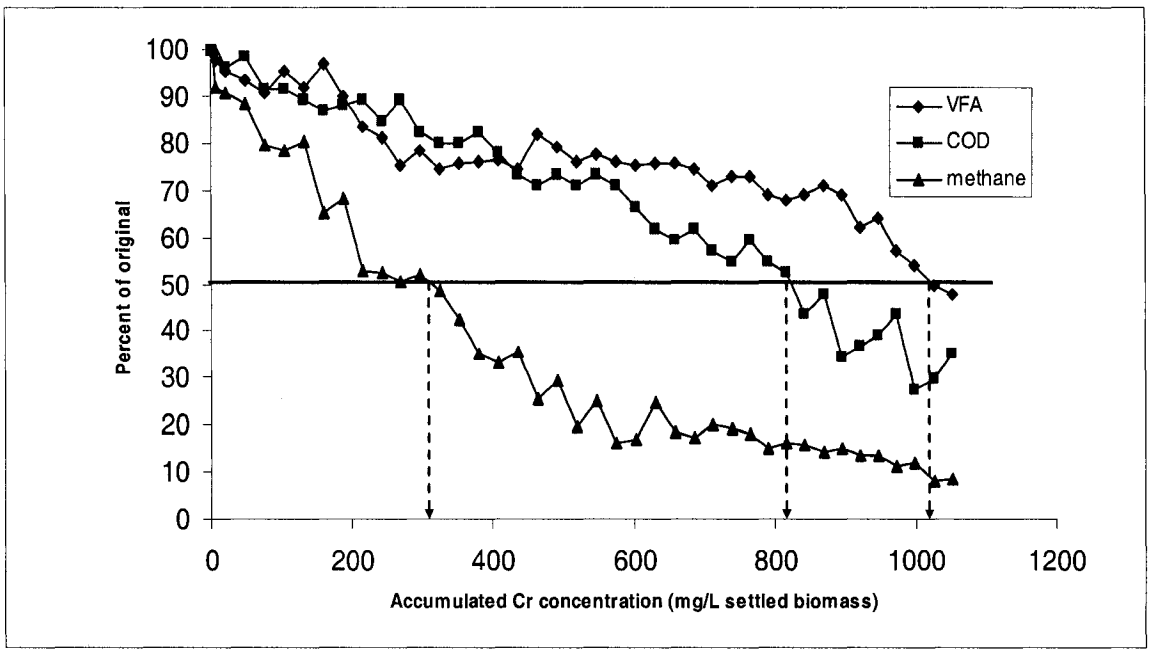

Figure 4-34. Relative percent reduction of COD removal, VFA concentration, and methane production versus accumulated chromium dose for reactor one.

Since percent reduction and percent inhibition are the same at the 50% level, Figure 4-34 can be used to estimate ID50 toxic indices. Based on COD removal for the ASBR experiment an ID50 of about 825 mg Cr/L settled biomass can be determined. It must be stressed that the "chromium dose" is an accumulated one.

Thus, an almost 3-fold resistance to Cr(VI) loading was obtained for biomass in the ASBR experiment when compared to the batch test data. The low stepwise dose regime may give bacterial cells in reactor one, that were damaged by the oxidative action of Cr(VI), a relatively better chance to recover when compared to the batch test biomass.

In a study using Cr(III), it was found that a shock dose of 500 mg Cr(III)/L total reactor volume resulted in process failure for anaerobic digestion system; however, a total concentration of 1140 mg Cr(III)/L, achieved by a low stepwise dose, was required to induce process failure (Alkan et al. 1996). In addition, very little difference has been found by some researchers between the toxicity of Cr(III) and Cr(VI) in anaerobic digesters, which was believed to be due to the rapid reduction of Cr(VI) to Cr(III) in the anaerobic environment (Alkan et al. 1996). Although, as mentioned earlier, no toxicity index such as an ID50 could be generated for Cr(III) using a batch test technique due to pH adjustment problems (section 4.1.1), a preliminary ASBR experiment using the same approach as that described in this study for Cr(VI), was conducted using Cr(III). The difference however, was that the pH of reactor one, which could be automatically controlled, was set to 4.7±0.1 so as to eliminate any initial precipitation of the added chromium nitrate. Only one trial run was performed in contrast to the Cr(VI) experiment which was performed in duplicate. The experiment was stopped after 25 one liter addition cycles of 25 mg Cr(III)/L, when Cr(III) levels in the effluent were greater than 1 mg/L. This amounted to a final accumulated dose of about

694 mg Cr(III)/L settled biomass, based on an initial settled biomass volume of about 910 mL for reactor one. The only monitored parameter was COD removal which was reduced by 40% at the final accumulated dose. The experiment was terminated at this point (30 days), unlike the Cr(VI) study which lasted for 42 days.

However, ignoring any effect due to pH in reactor one between the Cr(III) and Cr(VI), the findings also suggest little or no difference in the toxicity of Cr(III) or Cr(VI). Since a 50% reduction in COD was not obtained for the Cr(III) study, an ID40 values for Cr(III) and Cr(VI) based on accumulated dose can be calculated and compared. An ID40 value of about 694 mg Cr(III)/L settled biomass can be estimated based on the Cr(III), compared to the 681 mg Cr(VI)/L settled biomass obtained for the Cr(VI) study. This similarity in toxicity suggests that biomass is able to recover from stepwise, low level loadings of Cr(VI) since it is well established that soluble Cr(VI), as the chromate anion, is more toxic than the relatively insoluble Cr(III) compounds (Srinath et al. 2001, Turick et al. 1996).

Analyzing the inhibition of methane production, obtained in the ASBR experiment, according to the SMA procedure presents many difficulties. First of all, the SMA test usually involves a one time addition of toxicant prior to determining methane production rates over a short period of time such as 1 to 6 days. Secondly, the substrate is generally enriched with respect to VFAs. Although methane production rate in reactor one is of interest, the main concern

in this study is not the toxicity of Cr(VI) to the methanogenic bacterial population per se. The goal is the removal and reduction of Cr(VI) in reactor one and the maintenance of methane production in reactor two. However, it is also desirable to extend the duration of metabolic activity in reactor one with respect to VFA production to take advantage of the large sorption capacity of the cheese whey biomass for Cr(VI). Since methane production, COD removal and the content of VFAs in reactor one are interrelated with respect to the metabolic activity of the biomass in reactor one, some index of toxicity using ID50s for COD removal, methane production rate, and relative VFA content (as an estimate of relative net VFA production) may be useful in elucidating the overall toxicity of Cr(VI) loading in reactor one and be a practical tool in scaling up the laboratory scale ASBR study to pilot or plant size operations.

The ID50 for COD removal was already determined as 825 mg Cr/L settled biomass. If the reduction in methane production relative to day zero versus accumulated dose of chromium (Figure 4-34) is taken into account, an ID50 of about 320 mg Cr/L settled biomass can be estimated. Similarly, an ID50 of about 1125 mg Cr/L settled biomass based on the percent reduction in VFA content relative to day zero can be estimated. Based on these individual 50% inhibition levels for accumulated chromium in reactor one, an average $ID50_{avg}$ of 757 mg Cr/L settled biomass can be generated. At this accumulated level VFA content will be greater than 70% of the initial day zero content and COD removal will be greater than 50%. Methane production will be about 20% of the initial production

rate. Even though the addition of effluent from reactor one to reactor two was terminated before accumulated chromium levels reached the $ID50_{avg}$ value of 757 mg Cr/L settled biomass, consideration of the relative VFA content of the effluent in reactor one at this accumulated chromium loading indicates that it would be a more than suitable feedstock for reactor two. However, it is known from the ASBR experiment that at this level of chromium accumulation, suspended chromium will occur in the effluent of reactor one at levels of about 1 mg/L. Again, the goal is to maintain reactor two under negligible chromium loading, and these results suggest some fine tuning of operational parameters such as reduced mixing of the biomass in reactor one or an intermediate filtration or settling operation is necessary to remove suspended chromium.

4.6 Hexavalent Chromium Removal Using Biomass and Biomass Supernatant Components

The experimental protocol described in section 3.4.5 was used to determine the reduction or removal capabilities of different "compartments" of reactor one such as the biomass granules, and the extra-granular liquid phase. The results of this experiment are given in Table 4-5. The percent removal for Cr(VI) was based on the difference between the distilled water-Cr(VI) control and test Cr(VI) concentrations after the 24 hour incubation period. Reduced Cr(VI) was calculated based on the difference between total chromium and Cr(VI) measurements.

Table 4-5. Cr(VI) removal by granular compartment and reduction by extra-granular compartment.

Biomass	Initial Cr(VI) (mg/L)	Amount of Cr(VI) removed (mg/L)	Percent Removal
Distilled water (blank)	560	n/a	n/a
Unwashed biomass	111	449	80
Washed biomass	168	392	70
Liquid phase	**Initial Cr(VI) (mg/L)**	**Amount of Cr(III) produced (mg/L)**	**Percent reduction**
Distilled water (blank)	36.6	n/a	n/a
Unfiltered supernatant	12.7	23.9	65
Filtered supernatant	17.2	19.4	53

These results indicate that the liquid fraction of the settled biomass is capable of removing Cr(VI). In the case of the supernatant filtered through the 0.45 μm filter, it can be said that the removal of Cr(VI) involved its reduction to Cr(III). Since there is no suspended particulate matter for Cr(VI) to sorb to, the removal must be facilitated by the reduction of Cr(VI) to Cr(III) with subsequent precipitation of an insoluble, trivalent chromium compound. Experiments with simulated wastewater containing either sucrose or acetate showed no ability to reduce Cr(VI) during a 24 h incubation period. As far as the unfiltered supernatant is concerned it can be assumed, based on the filtered supernatant result, that at least 53% of the removal of Cr(VI) involved chemical reduction to Cr(III). The other 12% would most likely involve sorption of the Cr(VI) to suspended organic material; however, it is unlikely that it would remain as Cr(VI), or as the chromate species, since as a powerful oxidant it would oxidize the organic material it was initially sorbed to, resulting in its own reduction to Cr(III).

The results for the biomass granules also demonstrate its ability to remove hexavalent chromium from the liquid phase. The unwashed biomass exhibited slightly more (10%) ability to remove Cr(VI). This is most likely due to suspended organic material loosely adhering to the granule surface, which was removed during washing. However, the necessary ratios of added Cr(VI) to the liquid phase and biomass phase volumes in order to obtain residual and removed or reduced Cr(VI) in the supernatants differed greatly. Based on volume of settled biomass and volume of supernatant, the settled biomass was exposed to about 500 mg Cr(VI)/L settled biomass, and the supernatant had a Cr(VI) concentration of about 37 mg Cr(VI)/L. Thus, the relative exposure level is about 14 times more for the settled biomass granular compartment when compared to the supernatant compartment. It can be concluded that the granular biomass has a much greater capacity to remove Cr(VI) by sorption than does the supernatant which would facilitate removal by reduction of Cr(VI) to Cr(III), followed by precipitation of insoluble Cr(III) compounds.

4.7 Speciation of Cr(VI) on the Biomass

A selective sequential extraction (SSE) was carried out on the spent biomass from reactor one. The distribution of heavy metals in sludge is related to the type and sequence of chemical extractants used in the procedure. Others using the same SSE procedure for anaerobically digested sludge found that chromium is mostly incorporated into organic matter (65%). The remaining 35% was bound to Fe/Mn oxides (15%) and residual (20%) (Marchioretto et al. 2002). The SSE in

this study found that 45% of the chromium was recovered with what is referred to as the (hydr)oxide fraction and which corresponds to the Fe/Mn oxides fraction in the abovementioned study. This chromium fraction is believed to be a mineral fraction composed of Cr(III) oxides or hydroxides associated with other mineral oxides in the fraction. The remaining 55% of the recovered chromium was associated with the organic matter fraction of the biomass. This chromium fraction is believed to be incorporated into organic matter through complexation (Cr(III)-organo complexes). No soluble, exchangeable, carbonate or residual chromium fractions were found.

These results imply that soluble Cr(VI) was completely reduced to insoluble Cr(III). This Cr(III) was almost equally distributed between insoluble mineral (hydr)oxides and Cr(III)-organo complexes. It is interesting to note that no soluble or exchangeable chromium was found. This confirms the efficacy of the cheese whey biomass as a sorption-reducing agent for Cr(VI). These results also imply a fairly simple model for the ultimate fate of Cr(VI) in reactor one of the two-phase ASBR system involving sorption of Cr(VI) followed by reduction to Cr(III). The fate of hexavalent chromium in reactor one is discussed in Chapter Five.

4.8 Chromium Leachability from the Biomass

Since the spent biomass from reactor one will be contaminated with chromium, disposal methods should be in compliance with hazardous material disposal guidelines. Spent biomass, removed from reactor one after 42 days had an

average settled volume of about 810 mL and had a calculated accumulated amount of chromium of about 950 mg or 1173 mg Cr/L settled biomass. Spent biomass from reactor one (12 mL settled volume) gave a value of 1140 mg Cr/L settled biomass ± 3% (triplicate) for the acid digested control for total chromium in the sequential extraction experiment. Thus, an excellent correlation between the calculated and the experimental amount of sorbed chromium was obtained. Since the biomass is about 97% water by weight this means that there will be about 38 g Cr/kg dry weight of biomass. Digestion of dried biomass from reactor one (2 g) gave a value of 36 g Cr/kg dry weight ± 9% (in triplicate).

In order to examine the potential for chromium mobilization, leaching tests were conducted. Levels of chromium in the leachate were < 0.01 mg/L (based on the detection limit for the spectrophotometric determination of Cr(VI) by the diphenylcarbazide method) for both wet and dry chromium-contaminated spent biomass from reactor one after a five month leaching period. The EPA toxicity characteristic leaching procedure (TCLP) has established a leaching limit of 5 mg/L of total chromium from contaminated soil (EPA 1997). The non-leachability of chromium from the spent biomass is in keeping with the results obtained from the SSE experiment which indicated that 45% of the chromium was associated with insoluble mineral (hydr)oxides and the other 55% as chromium-organic complexes and organic mineral aggregates. No exchangeable or soluble chromium was found.

Although chromium leaching from the biomass was only studied for five months, the results indicate that the spent biomass, whether wet or dried, can be potentially disposed of in a landfill where suitable precautions are taken to protect surrounding, underlying groundwater. Such precautions would involve a properly lined landfill or internment in soil with a low redox potential, suitable pH, or low manganese oxide content, since it is known that manganese containing soils can potentially oxidize Cr(III) to Cr(VI) under low pH conditions (Bartlett and James 1979). Considering the ability of the spent biomass to retain chromium under conditions of relatively low pH, the feasibility of using the two-phase ASBR system as a means for treating Cr(VI)-contaminated industrial effluents is once again borne out.

4.9 Concluding Remarks

Batch tests indicated that methanogenic bacteria in the phase I optimized biomass were more resistant to Cr(VI) exposure than that in the phase II optimized biomass. A previously explained, a toxicological index was devised based on "dose" rather than concentration, as sorption of Cr(VI) to biomass granules was complete and rapid at the Cr(VI) concentrations investigated. Sorption isotherms for viable and non-viable biomass followed the Langmuir model. Viable biomass exhibited greater sorption capacity and also showed evidence of a biomediated sorption component based on a comparison of Cr(VI) biosorption in control and metabolically inhibited biomass. No apparent benefit in

the use of Cr(VI) acclimated biomass was observed, and non-acclimated, phase I optimized biomass was used in the ASBR experiment.

The ASBR study demonstrated feasibility of using a two-reactor system where removal of chromium through reduction and sorption was initiated in the first reactor, allowing methanogenesis to proceed in a chromium-free environment in the second reactor. Based on ASBR data for COD removal in reactor one a toxicological index based on accumulated dose was developed. Spent biomass from reactor one exhibited strong binding of Cr(III) as witnessed by leaching studies, demonstrating the feasibility of landfill disposal. The SSE experiment found that all sorbed Cr(VI) was reduced to Cr(III) and was almost equally distributed between Cr(III) hydroxides and organo-Cr(III) complexes. The results of sorption studies and the SSE experiment allow for the development of a model for the fate of Cr(VI) in reactor one of the ASBR system.

Chapter Five

FATE OF CHROMIUM UNDERGOING BIOSORPTION

5.1 Proposed Mechanism for Cr(VI) Removal

Although the equilibrium experiment determined that essentially all the added hexavalent chromium is sorbed to the biomass within 15 minutes of contact, and the sequential extraction found that the sorbed chromium occurred as insoluble Cr(III) hydroxides and organo-Cr(III) complexes, understanding the mechanism of hexavalent chromium removal in reactor one of the ASBR treatment system presents some difficulties due to the complex nature of the system. Reactor one can be compartmentalized into a non-granular, liquid phase, which would initially contain the soluble Cr(VI), and a granular biomass phase, which would eventually be the phase where chromium, as Cr(III), would accumulate. The non-granular compartment would contain a complex mixture of soluble organic and inorganic material, as well as suspended organic material. The granular phase would be comprised of a large mixed population of bacterial cells, essentially held together with a large amount of extra-cellular organic material. The granular phase can be further compartmentalized into an intra-cellular and extra-cellular phase. Considering the nature of hexavalent Cr(VI) and its possible interactions with the compartments of reactor one, delineating what actually occurs between its introduction as a soluble Cr(VI) compound and its final occurrence as insoluble Cr(III) associated with the granular phase presents a challenge. In

order to attempt to unravel the fate of the added Cr(VI), its possible interactions with the components of reactor one must be considered.

Hexavalent chromium was introduced into reactor one as soluble potassium chromate (K_2CrO_4). The most commonly encountered Cr(VI) anions are the bichromate $(HCrO4)^{-1}$, chromates $(CrO_4)^{-2}$ and dichromates $(Cr_2O_7)^{-2}$, which are both powerful oxidants. When in contact with organic matter, chemical oxidants such as the chromate anion can oxidize organic matter while concomitantly being reduced. Studies have shown that organic matter can chemically reduce the hexavalent chromium of chromates to trivalent chromium (Wittbrodt and Palmer 1995, Tzou et al. 2003, and Bolan at al. 2003). Chromium is believed to form organo-Cr(III) complexes with organic matter in a two step process where Cr(VI) is first reduced to a reactive Cr(III) intermediate, by compounds acting as electron donors, and then subsequently forms coordinated complexes with organic matter. The chemical cross-linking of hydrolyzed polyacrylamide by Cr(III), which is produced in situ by the reduction of Cr(VI) by thiourea, is a well-known example of physico-chemical reduction of Cr(VI), followed by the formation of organo-Cr(III) complexes. The cross linking is believed to occur through the carboxylic functional groups of the polyacrylamide (Maxcy et al. 1998). In addition, Cr(VI) can be chemically reduced to Cr(III), forming insoluble Cr(III) hydroxides, by ferrous iron (Fe^{2+}), which is concomitantly oxidized to ferric iron (Fe^{3+}) in the process. This redox reaction is favoured by the absence of dissolved oxygen (Fendrof and Li, 1996). So far only the chemical reduction of

Cr(VI) has been discussed, but in viable biological systems, biomediated or biochemical reduction of Cr(VI) is also possible.

It is known that organo-Cr(III) complexes can result from biomediated, or enzymatic reduction of Cr(VI). Hexavalent chromium is the final electron sink in biomediated redox reactions involving low molecular weight organic electron donors. The trivalent chromium formed will undergo chemical complexation with organic material as discussed previously with respect to chemical reduction. This process could occur in the extra-cellular component via excreted enzymes (exozymes) or in the intra-cellular component by cytoplasmic enzymes (endozymes) (Laxman and Moore, 2002, Michel et al. 2001, and Rege et al. 1997). Immobilized bacterial cells using lactate as the electron donor can enzymatically reduce Cr(VI) to insoluble, and precipitated Cr(III) compounds (Tucker et al. 1998). Hexavalent chromium that is actively taken up by cells can, after biomediated reduction, form organo-Cr(III) complexes with DNA, in addition to other biomolecules, resulting in the chemical cross-linking of the DNA molecules. This is believed to be the mechanism by which the mutagenic and carcinogenic properties of hexavalent chromium are expressed (O'Brien et al. 2001). In addition to organo-Cr(III) complexes, insoluble Cr(III) hydroxides are also produced during biomediated reduction of hexavalent chromium (Xun et al. 2004). Biomediated reduction of Cr(VI) to Cr(III) is well documented in anaerobic digestors (Srinath et al. 2001 and Alkan et al. 1996).

Although essentially all the added Cr(VI) is recovered with the biomass granules as Cr(III) hydroxides and organo-Cr(III) complexes, it seems possible, given the above examples of both chemical and biochemical reduction of Cr(VI) to Cr(III) and the composition of the liquid phase that some reduction could occur in the non-granular compartment of reactor one followed by precipitation of insoluble Cr(III) compounds unto the biomass granules. Iron-reducing bacteria found under anaerobic conditions can reduce ferric iron and produce relatively large amounts of ferrous iron; especially in the presence of acetate acting as the electron donor (Rioux and Fortin, 2003).

Since ferric iron was a component of the simulated wastewater, the occurrence of ferrous iron in the liquid phase of reactor one is more than likely given the anaerobic conditions and the ubiquitous occurrence of iron reducing bacteria in anaerobic bacterial populations, as well as the presence of acetate due to acidogenesis (Stabnikov et al. 2004, Rioux and Fortin 2003). Thus the potential to reduce Cr(VI) to Cr(III) hydroxides by a redox reaction with ferrous iron is possible in the non-granular liquid compartment. It should be made clear at this point that when the non-granular phase is referred to, this is the bulk liquid compartment, which does not include water held within the biomass granule.

An attempt was made to estimate the contribution of the liquid or non-granular compartment with respect to Cr(VI) reduction with an experiment using both filtered and unfiltered liquid supernatant from phase I optimized biomass (section

4.6). The filtered supernatant, which was prepared by filtering supernatant through a 0.45 μm filter, represented the contribution made by the soluble fraction. The unfiltered supernatant represents the contribution due to both soluble and suspended material. Although the filtered and unfiltered supernatants were able to reduce 53% and 65% of the added Cr(VI) after 24 hours, in the case of the unfiltered supernatant this represented only about 25 mg Cr(VI)/L liquid phase or extra-granular compartment (Table 4-5). In the same experiment it was also found that biomass granules, that were washed several times with distilled water, were able to remove 70% of the Cr(VI) from the liquid phase during the same 24 hour period. This represented about 360 mg Cr(VI)/L settled biomass or granular compartment. After the withdrawal phase from reactor one, an average of about 0.15 L of the liquid phase compartment remained inside the reactor, thus based on the above, this volume could account for the reduction of about 3.8 mg Cr(VI) to Cr(III).

Since no Cr(III) was found in the liquid phase, at least for the first 24 days of operation for reactor one, it should be assumed that the reduced chromium in the supernatant precipitated on or sorbed to the biomass granules. Based on a settled biomass volume of about 900 mL in reactor one, removal of about 324 mg Cr(VI) can be estimated. Based on total removal by the two compartments, about 99% of chromium removal is due to the granular compartment can be estimated. Although the above estimates were based on the original phase I optimized biomass, it is possible that a change in the non-granular liquid compartment

components responsible for any Cr(VI) reduction will occur over the course of the ASBR experiment, as might the removal capacity of the granular compartment, it appears that the reduction of Cr(VI) in the liquid compartment is negligible. Certainly, chemical reduction in the non-granular phase can be ruled out as a significant factor in Cr(VI) removal since if it occurred it would be responsible for about 1% of the overall removal.

But what about biochemical or biomediated reduction of Cr(VI)? In a sense an artificial situation was created in the above experiment, where ongoing biomediated reduction, as a result of secreted enzymes capable of redox reduction of Cr(VI), has been possibly compromised. In other words, the extent to which biomediated reduction would have occurred in the liquid phase had it been in constant contact with the viable biomass granules is not known. However, it is certain that secreted enzymes (exozymes) were components of the unfiltered liquid phase in the aforementioned experiment, and would have been able to continuously reduce Cr(VI) to Cr(III) if capable of mediating such redox reactions.

Thus, it can be concluded that any ambient amount of such enzymes at the time of Cr(VI) addition to reactor one had a negligible contribution to Cr(VI) reduction. However, is the possible secretion of more enzymes, over and above what was already there, to such an extent so as to result in significant levels of Cr(VI) reduction likely? Given that the removal of Cr(VI) was swift, this seems unlikely since these enzymes would presumably have to be synthesized or if available

would be secreted into the intra-granular space and then diffuse into the bulk liquid of reactor one. As mentioned above, the equilibrium experiment indicated that within the time interval of 0 to 15 minutes (since the first sample was taken at 15 minutes) essentially all the Cr(VI) was removed by the biomass granules. In addition, the ratio of settled biomass volume to liquid phase in the ASBR experiment was much greater than in the equilibrium study, and the exposure level to Cr(VI) was much less. Thus, an even swifter uptake of chromium would be expected in the ASBR experiment when compared to the equilibrium study. As mentioned above, any secreted enzymes capable of redox mediated reduction of Cr(VI) would first occur in the greatest concentrations in the trapped liquid of the granule or the intra-granular space, and any reduced Cr(VI) would presumably precipitate as Cr(III) hydroxides or form organo-Cr(III) complexes within the granule itself. Although extra-granular reduction cannot be ruled out it seems unlikely that any significant reduction of Cr(VI) to Cr(III) would occur in the extra-granular compartment.

The removal of Cr(VI) by biomass granules from the liquid phase of reactor one would thus appear to result from both biosorption and bioaccumulation. In general several physico-chemical mechanisms for the removal of heavy metals using biosorbents are possible. Some examples are ion-exchange, sorption (adsorption and absorption), complexation, and precipitation. With viable biosorbents metal removal is mainly achieved through adsorption and complexation (Tchobanoglous et al. 2003). As mentioned earlier both

biochemical and chemical reduction to Cr(III) hydroxides and/or reduction to Cr(III) followed complexation with organic matter are possible in the case of Cr(VI) interacting with the biomass granules. The first stage in this interaction would be the initial sorption of Cr(VI) to the biomass granules. It is known that high molecular weight extra-cellular polymers of anaerobic biomass flocs can provide many functional sites where binding of cations and anions can occur (Alkan et al. 1996).

Thus some of the initial sorption of chromate anion to the biomass granules could involve electrostatic attraction such as found in ion exchange processes as well as coordinated complexes with functional groups of the extra-cellular organic material which would also include the cellular membranes of the bacterial populations. However, due to the oxidizing nature of the chromate anion it is unlikely that it would remain bound or exchangeable as the chromate species. Indeed the sequential extraction found no exchangeable or soluble chromium associated with the spent biomass, and only Cr(III) was recovered. In addition, active cellular uptake or bioaccumulation of Cr(VI) by the bacterial populations is also possible as referred to earlier in this discussion. Thus it is likely that Cr(VI) is both initially sorbed to extra-cellular organic material and taken up intra-cellularly by viable bacteria. Cr(VI) adsorbed to extra-cellular organic material could then undergo both chemical and biochemical, or bio-mediated reduction, to Cr(III) hydroxides or organo-Cr(III) complexes. Cr(VI) that was actively taken up by bacterial cells could then also undergo the same reduction reactions as those

which occurred in the extra-cellular component. Thus, with respect to the extra-cellular compartment, physico-chemical adsorption of Cr(VI) to extra-cellular organic material followed by chemical and biomediated reduction is possible. As far as the intracellular compartment is concerned it is possible to have biomediated uptake of Cr(VI) followed by both chemical and biochemical reduction. The reduced chromium compounds can be Cr(III) hydroxides or organo-Cr(III) complexes. With respect to the contribution of biomediated as opposed to physico-chemical reduction-removal, the experiment with metabolically poisoned biomass indicated that at least 25% of the reduction-removal was biochemical in nature.

5.2 Concluding Remarks

Although a precise account of the fate of added hexavalent chromium to reactor one, was beyond the scope of this study, the experimental data from the ASBR experiment, interpreted in the light of this research, allows us to formulate a reasonable scheme depicting the possible fate of Cr(VI) undergoing treatment in reactor one. In this scheme soluble chromate is introduced into the extra-granular liquid phase of reactor one whereupon it undergoes complete and rapid sorption to the biomass granules. Reduction of sorbed Cr(VI) can proceed in the extra-cellular compartment of the granules through chemical or biomediated pathways to insoluble Cr(III) hydroxides or organo-Cr(III) complexes. Intra-cellular uptake of chromate can also result in the same aforementioned reduction pathways. This scheme is illustrated in Figure 5-1.

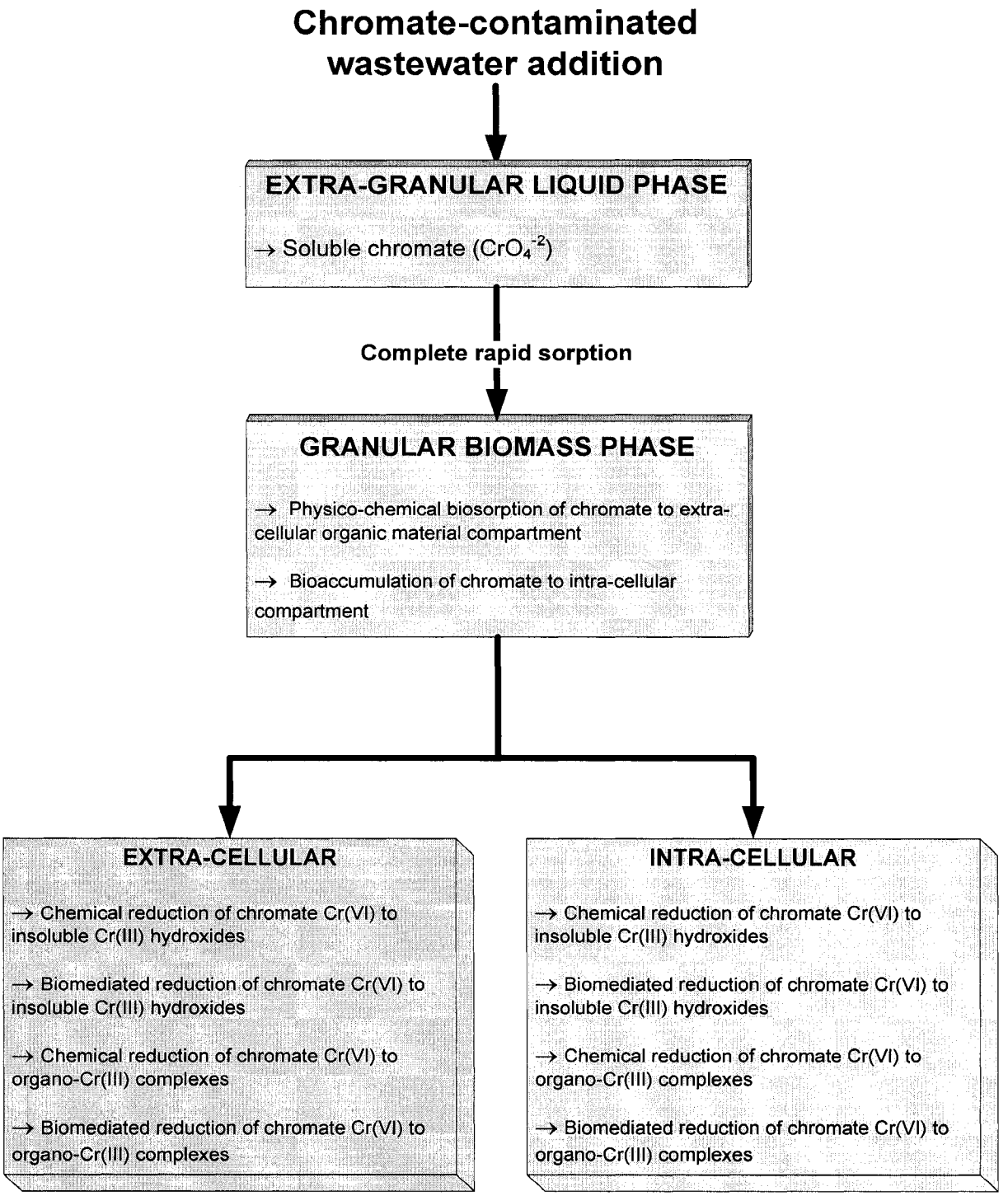

Figure 5-1. Scheme of the fate of Cr(VI) added to reactor one

Chapter Six

SCALE-UP MODELS

6.1 Modeling Based on Batch and ASBR Experiments

In order to design future laboratory or pilot scale two phase ASBR systems for treating wastewater streams containing heavy metals, the sorption potential, measured by q_{max}, and the toxicity of the metal contaminant, measured by an ID50 for example, must be considered. The q_{max} value, based on the Langmuir model was 4.74 mg Cr(VI)/mL settled biomass. Thus one liter of settled biomass could potentially sorb 4.74 g of Cr(VI). However, the toxicity of the sorbate if the system is to function as a metabolically active one must be taken into account; at least for a specified period, until accumulation leads to failure of the system. An estimated ID50 for the cheese whey biomass based on COD removal was 283 mg Cr(VI)/L settled biomass. Even though the settled biomass is about 25% extra-granular water, the LD50s were calculated based on settled biomass volume for the sake of convenience. The original batch test design used the water associated with settled biomass to adjust final concentrations of added chromium concentrations; however, the ID50 data are based on the amount of Cr(VI) added in mg and the amount of biomass. Since operational parameters for the amount of biomass would involve settled biomass volume, it would seem unnecessary to transform data to dewatered biomass volume (estimated by centrifuging biomass granules).

In the case of Cr(VI) the limiting loading factor is the ID50 and not the q_{max} value, since it is about 17 times greater. For any metal where q_{max} is less than the lethal index value, such as the ID50, the q_{max} would be the limiting loading factor. Thus, a simple design equation as an aid to determine the amount of wastewater that can be treated using a laboratory scale version of a two-phase ASBR system can be developed. It is common practice to use a safety factor when applying LD50s or ID50s; however, since a daily concentration of Cr(VI) of at least 1/10 of the ID50 is applied, and hexavalent chromium reduction to trivalent chromium will occur, using the LD50 without modification by a safety factor was considered to be a good initial range finding method for laboratory scale or pilot scale studies. Heavy metals which undergo little or no change in speciation to a lower toxic form may have a dilution factor applied so that a level of 50% mortality, for example, is not achieved too rapidly. A design equation for reactor one in a two-phase ASBR can be set up using the following generalization:

[Maximum allowable sorbed chromium] – [Total chromium input] = 0

or stated mathematically for the case where the ID50 ≤ q_{max} as:

$$[ID50 \times V_B] - [V_C \times N \times C_{in}] = 0 \qquad \textbf{6-1a}$$

or for the case where the q_{max} ≤ ID50 as:

$$[q_{\max} \times V_B] - [V_C \times N \times C_{in}] = 0 \qquad \textbf{6-1b}$$

where, $ID50_{Cr(VI)}$ = mg Cr(VI)/L settled biomass

q_{max} = mg Cr(VI)/L settled biomass

V_B= volume of settled biomass (L)

V_C= cycle volume in liters (L/cycle)

N = number of cycles

C_{in}= concentration of Cr(VI) in mg/L_W

In the above equation the ID50 necessarily refers to the accumulated concentration when the protocol requires more than one cycle of Cr(VI) addition. Rearranging the above equations allows determination of the number of addition cycles for the batch reactor when the accumulated concentration equals the specified ID50 or q_{max}. For the number of wastewater addition cycles in a two-phase ASBR the equations for reactor one become:

$$N = \frac{ID50 \times V_B}{V_C \times C_{in}} \qquad \textbf{6-2a}$$

or,

$$N = \frac{q_{\max} \times V_B}{V_C \times C_{in}} \qquad \textbf{6-2b}$$

In the laboratory scale ASBR experiment about 24 one liter simulated wastewater addition cycles of 25 mg Cr(VI)/L to one liter of settled biomass for reactor one until trace amounts of chromium were found in the effluent is achieved. In addition to the above 24 liter additions, 4 one liter additions, of a gradually increasing Cr(VI) concentration, were also added (see section 4.5) for a total accumulation of 681 mg Cr/L settled biomass, based on an initial settled biomass volume of 900 mL. Although the total accumulation could have been based on the final settled biomass volume of 810 mL, yielding a total accumulation of 757 mg Cr/L settled biomass, the more conservative accumulation level was chosen; thus, in modeling the system initial settled volumes will be used. Applying equation 6-2 an estimated between 11 and 12 one liter addition cycles until accumulated chromium would have reached the average ID50 value can be obtained. However, the ID50 value was obtained for a two-day high level exposure of Cr(VI) which would tend to overestimate the toxicity when compared to the accumulated low level dose protocol.

The ID50 in the ASBR experiment for the COD removal based on accumulated dose of chromium is 825 mg/L settled biomass. This amounts to about 33 addition cycles based on calculated chromium accumulation using equation 6-2. If the $ID50_{avg}$ value of 757 mg Cr(VI)/L settled biomass based on methane production is used, relative VFA content, and COD removal, about 30 addition cycles can be calculated. The amount of wastewater treated based on the ID50 for COD removal is very close to that obtained when the $ID50_{avg}$ value is used.

Since COD determination is a relatively simple procedure, and COD removal efficiency incorporates both methane production and net VFA production, it seems to be an ideal parameter with which to assess the toxicity of Cr(VI) or other toxicants, especially since very little discrepancy in the amount of wastewater treated when the ID50 for COD removal or the $ID50_{avg}$ are used. However, after 12 addition cycles there was no chromium detected in the effluent of reactor one; but, after 33 addition cycles, chromium levels in the effluent reached levels of about 2 mg/L (Figure 4-32).

In the ASBR experiment chromium was detected as Cr(III) in the effluent of reactor one after about 24 addition cycles. Since a sorption level of only 681 mg Cr(VI)/L biomass was obtained before chromium was found in the liquid phase of reactor one after 28 days, compared to the maximum theoretical sorption level of 4700 mg Cr(VI)/L settled biomass, obtained from the 24 hour sorption study, the operational parameters of a laboratory or pilot scale experiment should be taken into account. For instance, biomass granules in reactor one were subjected to constant mixing at 50 rpm. Such mixing can result in the breaking away of small pieces of biomass granules which persist in the liquid phase as suspended material; which would account for the occurrence of chromium (associated with suspended particulate matter) in the effluent in reactor one after about 28 days of mixing. In addition to constant mixing, the increasing toxic effect of accumulating chromium could compromise the integrity of bacterial cells which comprise the granules. As discussed earlier, a slight increase in soluble COD at exposure

concentrations of 446 mg/L and higher was consistently obtained in the batch test studies. As argued earlier, this was most likely due to a slight breakdown of the granules cellular material due to the oxidizing, toxic effect of Cr(VI). About a 7% increase in soluble COD when compared to the control value was observed for a Cr(VI) exposure level of 446 mg/L. Filtration of the effluent from reactor one using a 0.45 μm filter indicated that the chromium was indeed associated with suspended matter.

In order to develop a two-phase ASBR pilot scale system for the removal of Cr(VI), it is suggested that a simple batch test study generate a pertinent ID50 value(s) for comparison with the q_{max} value for the biomass in question. The simple one or two day batch test can be used to determine the sensitivity of the biomass in question to Cr(VI) loading, and would represent a worst case scenario due to the high concentration, acute dose protocol. The batch test study would thus be conservative in that it will generate lower ID50 values; thus, overestimating the toxicity of Cr(VI). Using equations 6-1a or 6-1b will allow the investigator to at least determine minimal amounts of wastewater that can be treated based on initial concentrations of Cr(VI). A subsequent laboratory scale experiment will allow an investigator to determine the ratios of wastewater to settled biomass or VSS, for example, used so as to scale-up to a pilot study. The laboratory scale study involving a low concentration, chronic dose protocol would be necessary to better gage the efficacy of the system, as well as determine the toxicity of Cr(VI) under stepwise low chromium loading conditions. Toxicity data

derived from the ASBR experiment, such as an ID50 based on accumulated dose, could be used in equation 6-2a to predict the amount of water treated depending on the amount of the biomass and the concentration of Cr(VI) in the influent for reactor one. This has obvious applications in assessing water treatment capacities for actual plant size operations.

Thus, rather than scale up directly to a pilot scale study for example, it is suggested that a laboratory scale batch experiment be implemented. As mentioned above, this in conjunction with a sorption study, would allow the investigator to determine the efficacy of the biomass, and indeed screen various biomass samples, for potential use. It must be said however, that the notion of a two-phase anaerobic unit operation as a means for optimizing methane production has been criticized (Krich et al. 2005). Some believe the concept suffers from a fundamental flaw as acidogens and methanogens depend on each other for optimal metabolic activity. Hydrogen and acetate, as well as other VFAs, are strong inhibitors of the metabolism of acidogens. The methanogens, which remove these products by converting them to CH_4, perform a necessary role in the overall process (Krich et al. 2005). Thus, it seems likely that although it might be possible to remove large quantities of Cr(VI) in reactor one, any "critical" reduction in methanogenesis in that reactor would have serious repercussions as far as VFA production was concerned. This discussion leads to some design considerations with regards to operating a two-phase anaerobic system for the removal of Cr(VI). First of all, the goal is not to necessarily maintain optimal

methane production in reactor one, it is to use the biomass as a sorbent for Cr(VI) while maintaining the necessary acidogenic activity for VFA production for as long as possible. This study found, using batch tests, that optimizing cheese whey biomass for phase I activity decreased the methane content of the biogas by only 25%, and reduced the CH_4/CO_2 ratio by just about 38% (Table 4-1) when compared to the non-optimized biomass. Thus, the phase I optimized system initially maintained a fair amount of methanogenic activity, which was borne out by the maintenance of VFA production in the ASBR experiment.

This criticism is of little concern in this study since phase II activity must be sequestered from the toxic effects of chromium, so there is no choice but to use a two-phase anaerobic system. Using one reactor, where the two-phases may be naturally separated to some extent, such as upflow anaerobic sludge blanket (USAB), where mixing is minimal, may be a feasible alternative, however; the phase II methanogenic populations could be readily exposed to suspended Cr(III), and possibly to Cr(VI) as well. In addition, any channeling of the Cr(VI)-contaminated influent would seriously compromise methane production in a one-reactor system. The two-reactor system allows for complete sequestering of the sensitive methanogens from the toxic effects of chromium. Also, removal of the spent chromium-contaminated biomass is readily facilitated by the use of a two-reactor system. Optimum removal of chromium-contaminated biomass would be more problematic in a one-reactor system, and any remaining chromium after spent biomass removal could potentially mix with the remaining phase II

population upon addition of fresh biomass to the reactor. In order to maximally remove chromium from the system it may be necessary to remove considerable amounts of phase II bacterial population. In addition, there would be no downtime for methanogenic phase activity in a two-reactor system, as opposed to a one-reactor system, where shutdown of the reactor would be necessary for removal of spent biomass. It should be stated that the above discussion contrasting a one-reactor and a two-reactor system is not based on actual studies, and comparing the feasibility of both systems could form the basis of a future study, however; the two-reactor system would appear to be a more attractive alternative based on the above discussion, and the bottom line is to protect the methanogenic population from any chromium exposure.

However, the question may arise as to why phase I optimized biomass was used in reactor one as opposed to non-optimized. First, the ID50s based on COD removal for each biomass was essentially the same (283 and 278 mg Cr(VI)/L settled biomass for phase I and non-optimized phase) and secondly, the methane content of the biogas produced by the non-optimized biomass in the batch test study exhibited a greater relative decrease when compared to the phase I optimized biomass. The methane content of biogas produced by phase I optimized biomass exhibited a 6% decrease at a Cr(VI) exposure level of about 56 mg/L when compared to initial values. The non-optimized system exhibited a 24% decrease at the same exposure level. Thus there seemed to be little advantage of using non-optimized biomass in the ASBR study.

Thus, if we wish to maintain optimal VFA production in reactor one it would seem necessary to preserve "some" methanogenic activity. Again since methanogenesis and acidogenesis work in tandem, as long as sufficient acidogenic activity is maintained in reactor one, despite decreases in methane production, satisfactory production of VFAs might be expected to continue. Indeed the VFA production data (Figure 4-27) show that they were maintained throughout the experiment despite the fact that methane production had dropped off by 80% of the initial rate after about 28 one liter addition cycles of Cr(VI).

Therefore, it would appear that if about 20% of the initial methanogenic activity is maintained, the net production of VFA will be maintained in the effluent of reactor one such that it will be a suitable feedstock or influent for reactor two. In other words, using an ID80 value for methane production, which was around 714 mg Cr/L settled biomass, in the ASBR experiment, would yield about 29 one liter addition cycles of 25 mg Cr(VI)/L using equation 6-2a. Again, this ID80 value gives similar amounts of wastewater treated when compared to that obtained for the ID50 for COD removal (33 one liter addition cycles) or $ID50_{avg}$ (30 one liter addition cycles). Thus, there does not seem to be much discrepancy between using the ID80 for methane production and the ID50 for COD removal. Since COD removal is readily determined, it would appear to be a good choice for evaluating overall toxicity to Cr(VI) loading in reactor one for scale up purposes. This type of data can only be obtained using results derived from a long term ASBR study, and should be a necessary prelude to any pilot scale study.

Even though it has been established that the chromium is in the form of the less toxic Cr(III) species in the effluent of reactor one, other work has shown Cr(III) to be toxic to the anaerobic digestion process (Alkan et al. 1996). Batch test experiments in this study with Cr(III) proved to be problematic due to the insolubility of Cr(III) under batch test conditions. Cr(III) solutions exhibited a thick milky appearance upon pH adjustment making precise administered doses difficult to achieve. When the Cr(III) stock solution was added to the biomass, immediate pH adjustment was required due to the extremely low pH of the stock solution (pH< 2). Any variation in pH adjustment time could result in variability of the measured toxic effect on the biomass due to pH alone. Using buffer solution to maintain required pH values proved to be not much better. Deviations from the target pH value were also observed and further pH adjustment was still required due to the relatively high Cr(III) concentration. Precipitation of Cr(III) of course still occurred in the buffered system. Another attempt using a target pH of 4.7 to avoid precipitation of Cr(III) also encountered similar problems due to the difficulty in manually adjusting the pH of the small volumes of liquid phase used in the batch test studies.

Although Cr(III) did have an adverse effect on the anaerobic digestion process in the batch test studies, the relative error between replicate test samples was very high (as much as 100%), and reliable results could not be obtained. However, the relative error between Cr(VI) replicate test samples was around 10%. It should be recalled that one preliminary ASBR experiment was performed using

Cr(III) since the pH controller of the unit could automatically adjust the pH of the reactor which was maintained at pH 4.7 to keep Cr(III) in solution. The results of this experiment showed little difference between the toxicity of Cr(VI) and Cr(III). Again it must be stressed that this study is primarily involved with the treatment of Cr(VI) using biosorption, and in fact a worst case scenario for the living biomass; direct exposure to Cr(VI) is being examined.

Another approach to estimate the potential amount of wastewater treated in pilot or actual plant size operations, using data derived from a laboratory scale experiment, is to use existing general equations for substrate utilization in batch reactors, and modify the rate constants based on accumulated chromium. Many of these equations describe first order reactions; for example, the general formula of substrate utilization for a batch reactor is as follows (Kuo 1998):

$$C_f = C_i \times e^{-k.t} \qquad \textbf{6-3}$$

Where, C_f is the final concentration (mass/volume).

C_i is the initial concentration (mass/volume).

k is the rate constant (1/time).

t is the residence time (time).

Assuming that the removal of COD in the ASBR experiment follows first order kinetics, a rate constant for COD removal can be estimated based on experimental results using daily initial influent and final effluent COD values. However, since chromium is building up in the system, and since it has a toxic effect, the rate constant in the above first order reaction formulation would in fact change over time. If the daily rate constant (k) over the complete 42 day interval versus accumulated chromium concentrations (mg Cr/L settled biomass) is plotted, the following graph is obtained (Figure 6-1).

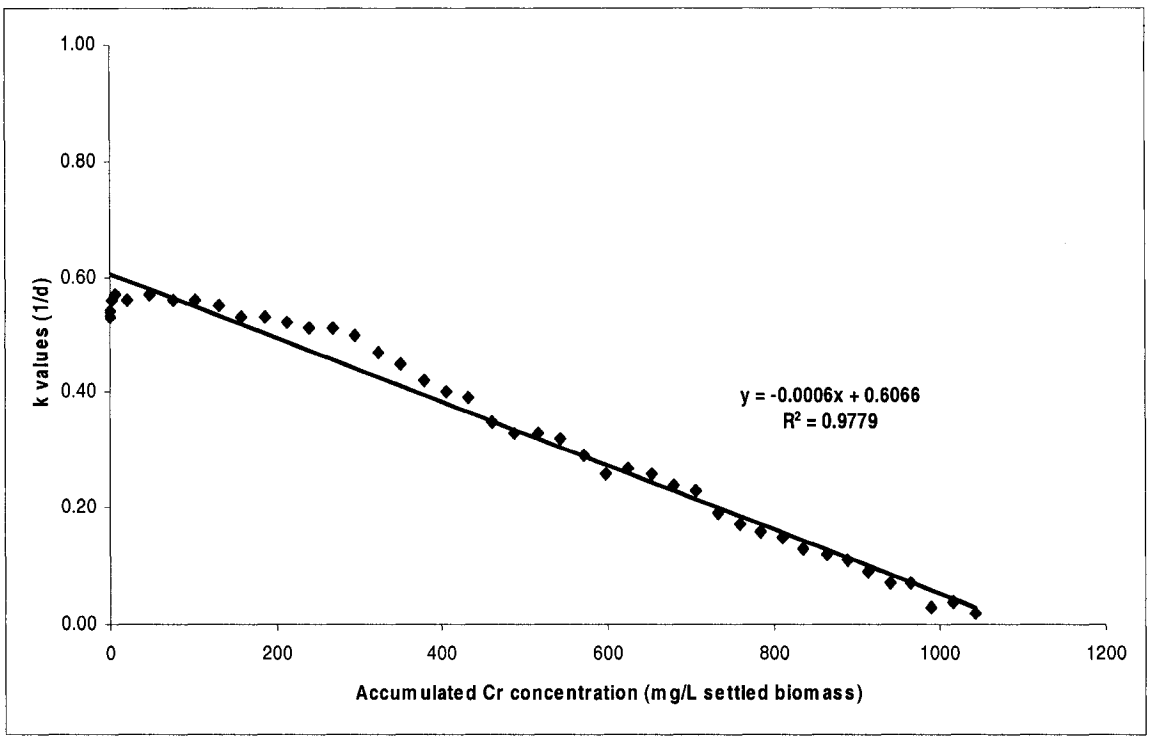

Figure 6-1. First order rate constant versus accumulated chromium

It can be seen from this figure that the rate constant as a function of chromium accumulation decreases with a slope of -0.0006 $L_{BM}.d^{-1}.mg^{-1}$ (where L_{BM} is liter

settled biomass). Due to the toxic effect of accumulated chromium in reactor one, the rate constant for equation 6-3 would change based on accumulated chromium levels. Since it is known that the chromium loading in the ASBR is such that essentially all the chromium is sorbed to the biomass granules, equation 6-3 can be modified by introducing a modified k based on chromium loading. Linearizing equation 6-3 gives:

$$LnC_f - LnC_i = -k.t \qquad \textbf{6-4}$$

Setting $$k = k_0 - k' L_{Cr} \qquad \textbf{6-5}$$

where; k' = is the slope of k values vs. C_{Cr} ($L_{BM}.d^{-1}.mg^{-1}$)

L_{Cr} = the accumulated chromium loading (mg/L_{BM})

k_o = the rate constant when $L_{Cr} = 0$ (d^{-1})

t = residence time (d)

k = modified rate constant (d^{-1})

Thus equation (6-4) becomes:

$$Ln(\frac{C_f}{C_i}) = -(k_0 - k' L_{Cr}) \times t \qquad \textbf{6-6}$$

For a batch reactor L_{Cr} (mg/L) is:

$$L_{Cr} = N \times (\frac{V_C}{V_B}) \times C_{Cr} \qquad \textbf{6-7}$$

where, N = number of addition cycles

V_C = volume of wastewater added per cycle (L/cycle)

V_B = initial volume of settled biomass (L_{BM})

C_{Cr} = the concentration of Cr(VI) in the wastewater (mg/L)

Inserting the values for L_{Cr} into equation 6-6 gives us:

$$Ln(\frac{C_f}{C_i}) = -[k_0 - k' \times N \times (\frac{V_C}{V_B}) \times C_{Cr})] \times t \qquad \textbf{6-8}$$

Rearranging equation 6-8 for the number of addition cycles gives the following:

$$N = \frac{[Ln(\frac{C_f}{C_i}) + k_0 \times t]}{[k' \times (\frac{V_C}{V_B}) \times C_{Cr}] \times t} \qquad \textbf{6-9}$$

Since the initial COD removal in the absence of chromium was on average about 44%, with a residence time of one day and a k value of -0.53 d^{-1}, a critical limit for COD reduction into equation 6-9 can be introduced; for example, setting a reduction of COD in reactor one of 0.5 relative to the control (44%), gives a value of 22% reduction or a C_f/C_i value of 0.88. Inserting this value into equation 6-9,

for a C_{Cr} value of 25 mg Cr(VI)/L, a V_W of 1 L, and a V_B of 0.9L, the following is obtained:

$$N = \frac{[Ln(0.88) + (-0.53day^{-1})(1day)]}{[(-0.0006L.day^{-1}.mg^{-1}) \times (\frac{1L.cycle^{-1}}{0.9L}) \times (25mg.L^{-1})] \times 1day}$$

$$N = 39\ cycles$$

It should be noted that the ID50 as well as the modified first order rate constant are specific values which depend on a variety of variables such as biomass type, pH, temperature, COD loading, agitation, etc. However, the method employed in their determination is a general one which can be applied to any anaerobic biomass involved in treatment of toxic metals where biosorption is a factor. A summary of the various estimated number of one-liter addition cycles (N) values based on the ASBR data is given in Table 6-1.

Table 6-1. Estimated one-liter addition cycles (N) of Cr(VI)-containing wastewater according to different modeling approaches.

Model	N
$ID50_{avg}$ using equation 6-2a	30
ID80 for CH_4 using equation 6-2a	29
ID50 for COD using equation 6-2a	33
Modified rate constant for 0.5 control COD removal equation 6-9	39
Experimental cycles at 1 mg Cr/L effluent levels	25
Experimental cycles at 2.5 mg Cr/L effluent levels	39

The N value (39 addition cycles) obtained using the first order rate constant model, which incorporates the COD removal at a reduced level of 50% relative to the control COD removal efficiency, is greater than the N value (33 addition cycles) obtained using the COD removal ID50 in equation 6-2a. However, the difference is not so great considering that the N value based on the ID50 was empirically determined and the N value based on the modified first order rate constant had evaluated the experimental data using a theoretical model. The values of N generated, by considering the ID50s for various parameters, range from 29 to 39 addition cycles. Obviously, the 39 addition cycles presents the best case scenario for the amount of water treated.

The ASBR experiment was terminated after 42 addition cycles, which represents about 39 additions of 25 mg Cr(VI)/L if the first four lower Cr(VI) concentration cycles are considered. At this point, the relative net VFA production was reduced by about 52% and COD removal by about 66%. Thus, it is still possible that reactor one could have remained in operation up until this point, however, no data are available for the performance of reactor two under the substrate loading (without chromium) conditions that would have resulted when COD removal and VFA production was reduced by more than 50%. However, as discussed earlier, in the absence of any accumulated chromium in reactor two there would not seem to be a problem with a sucrose substrate where about 30% (66% reduction of the initial control removal of 44%) of the overall COD had been removed;

some of which was transformed to VFAs, which resulted in a level that was about 48% of the initial VFA in the control effluent.

However, it is known from the ASBR experiment that adding Cr(VI) in conjunction with mixing will result in chromium in the effluent of reactor one, which will carry over to reactor two. Thus, when estimating the number of runs possible for a pilot scale or plant size operation for the removal of Cr(VI) using a two-phase ASBR system with mixing, one must decide between two alternatives. One is to use an intermediate settling basin or filtration operation between reactor one and two. In such a system the amount of water treated before reactor one became inoperable would be relatively greater; however, the operational cost considerations would be higher compared to the other alternative that is a system without any intermediate suspended chromium removal capability. Yet elevated costs due to increased removal rates for sludge or biomass in reactor one as well as lower chromium to spent sludge ratios resulting in larger sludge disposal volumes or increased pretreatment such as drying sludge prior to disposal could offset the initial attractiveness of the second alternative where no intermediate removal of Cr(III) carryover from reactor one to reactor two was used. In addition, the type of biomass used in the capacity of the biosorbent in reactor one is also important. Granular biomass such as the cheese whey biomass used in this study has good settleability which will minimize carry over of Cr(III) sorbed to the biomass. The settling characteristics of anaerobic sludge for example, are not as good as granular biomass and may result in greater carryover of Cr(III). Again,

laboratory scale experiments could readily screen types of biomass, not only for their sensitivity to Cr(VI) loading and sorption capacity, but also for their settling characteristics and potential for carryover.

Regardless of which two-phase ASBR system is chosen, equations such as 6-2a, 6-2b, or 6-9, based on some toxicological index such as COD removal efficiency can be extremely helpful in scaling up to pilot size or estimating the cost of plant size operations with respect to the amount of water treated and volume of sludge disposed etc. Observations associated with a laboratory scale test can also be of great value for designing pilot scale reactor systems. For example, the onset of suspended Cr(III) in reactor one well before failure may indicate the use of limited or no mixing in the first reactor where biosorption takes place in order to reduce Cr(III) carryover.

6.2 Examination of Treatment Alternatives Using the ASBR Model

In order to evaluate plant size operation using a two-phase ASBR system with cheese whey biomass for the removal and treatment of Cr(VI) from industrial wastewater, equation 6-2a can be used with the ID50 for COD removal determined in the ASBR experiment. For example if a typical average flow from an electroplating industry Cr(VI)-contaminated rinse water of about 50 m^3/d, containing an average concentration of about 10 to 13 mg Cr(VI)/L (United Nations 1982) is combined with a pig slaughterhouse wastewater stream having a COD of about 10,000 mg/L (Massé and Masse 2000) at a 1:1 ratio, a combined

wastewater flow of about 100 m^3/d with a COD of about 5,000 mg/L and Cr(VI) concentration of about 6.5 mg/L can be obtained. Using a settled biomass volume to wastewater cycle volume of 1:1 gives us a total reactor volume for reactor one of about 200 m^3 for hydraulic retention time of one day. Reactor two would have a volume of about 600 m^3 based on a settled biomass to reactor one effluent volume of 1:1 and a hydraulic detention time of 3 days. Using equation 6-2a, 127 treatment cycles is estimated, which represents 6,350 m^3 of electroplating wastewater treated, and corresponds to 82.6 kg of Cr(VI) treated, or 82.6 kg per 100 m^3 settled biomass. However, according to the sequential extraction, about 45% of the sorbed chromium is in the form of hydroxides. Therefore, assuming the remaining 55% to be complexed to organic matter as Cr(III) the inorganic chromium sludge weight can be modified from 82.6 kg to 119 kg. Since the settled biomass has a density of 0.9 g/mL this gives us a total sludge wet weight of about 209 kg wet weight.

The above example is an approximation and does not take into account other considerations such as reactor freeboard when estimating reactor volumes, but serves to illustrate how initial scale-up to working volumes of reactors can be achieved using the laboratory scale experiment biomass to cycle volume ratios. The important point of this example is to illustrate how equation 6-2a can estimate the amount of wastewater treated and Cr(VI) removed as Cr(III) regardless of the working reactor volumes, wastewater cycle volumes, or settled biomass volumes involved.

In order to gauge the relative efficacy of the above Cr(VI) reduction-biosorption treatment example it can be compared to a common chemical treatment method, such as chemical reduction by ferrous sulphate. Basic chemicals required for this treatment are: iron sulphate heptahydrate (16.03 kg required per kg chromium treated), sulfuric acid (6.01 kg required per kg chromium treated), and lime (9.48 kg required per kg chromium treated). The amount of sludge generated per kg chromium treated is: 6.01 kg of ferric hydroxide, 17.44 kg of calcium sulphate, and 1.98 kg of chromium hydroxide (United Nations 1982). Thus, a total chemical sludge wet weight of 25.43 kg per kg chromium treated can be calculated. Based on the above treated chromium of 82.6 kg in the two-phase ASBR example, this would give us a total sludge wet weight of about 2100 kg. In addition to insoluble chromium hydroxide, the other components of the sludge can also be considered as toxic chemical waste.

Thus, if the weight of sludge generated by the two-phase ASBR and chemical precipitation treatment are compared, we see that the chemical treatment generates more than 10 times the amount of sludge. In addition, the cost of the chemicals required for chemical precipitation of chromium should be considered in contrast to the biomass which is generated as a byproduct of the treatment of organic waste material. Also, chemical sludges are notoriously difficult to dewater, and although no concise study was made of the dewatering or drying characteristics of the spent biomass, it was observed that more than 90% of the moisture could be removed from the wet settled biomass after five days of

weathering in a fume hood at ambient temperature. Drying of chemical sludges, especially if heat is used can be very costly; however, the two-phase ASBR system generates methane as a byproduct which could also be used as a fuel source for drying spent biomass as well as maintaining elevated temperatures for the reactors (35°C). It should be noted that chemical precipitation for the removal of metals from inorganic wastewater streams can suffer from low removal efficiency depending on the solid separation techniques employed and further treatment such as supplementary pH adjustment and polymer addition may be required to enhance removal. Also metals held in solution by chelating agents such as ethylenediaminetetraacetic acid (EDTA) are difficult to precipitate (Federal Remediation Technologies Roundtable 2005). With respect to dewatering or drying spent biomass or chemical sludge, it is interesting to compare the amount of water in the spent biomass from reactor one with that for the chemical sludge in the above example. The biomass is approximately 97% water giving a total water content of about 97 kg with a dry solid content of about 122 kg (119 kg inorganic + 3 kg dry biomass). If the water content of the chemical sludge based on a typical average water content is estimated at about 85%, there is approximately 1785 kg of water and 315 kg of dry solid waste.

This comparison illustrates that dewatering of the spent biomass of reactor one would require much less energy since the amount of water in the chemical sludge is more than 18 times that of the spent biomass. Also, the dry solid waste from the chemical sludge is about 2.6 times greater than that derived from the

spent biomass. Thus compared to chemical treatment of inorganic effluents containing Cr(VI), the two-phase ASBR system represents a potentially attractive cost efficient and environmentally friendly alternative with respect to operation, amount of sludge generated, the nature of the sludge, and the absence of chemicals used in the process. Although other researchers (Tokunaga et al. 2003) have indicated a potential use of biological systems with respect to Cr(VI) remediation especially in the soil environment, a potential ecological problem, which has not apparently been addressed in the literature, may be of some concern. A problem could occur if soil microorganisms are artificially augmented in the presence of Cr(VI) or if viable chromium contaminated biomass, resulting from Cr(VI) exposure is interred in soil. Hexavalent chromium is a powerful mutagenic agent, and due to the short life cycle of microorganisms, has the ability to promote and produce mutated species with unknown consequences to the soil ecosystem. Thus, any viable populations of bacteria previously exposed to Cr(VI) could have high incidents of genetic mutation. It is therefore suggested that spent chromium-contaminated biomass from reactor one in a two-phase ASBR treatment system as outlined in this study, be heat-killed or dried prior to internment in soil or disposal in general so as to reduce the possibility of introducing significant amounts of genetically modified bacteria into the soil environment.

Chapter Seven

CONCLUSIONS AND CONTRIBUTIONS

7.1 Conclusions of the Results of the Study

The objective of this study was to develop a method for the removal of hexavalent chromium from industrial effluents using a two-phase ASBR. The batch test experiments involved in this study established the suitability of the cheese whey biomass as a biosorbent for Cr(VI), as determined by its toxicity; for the objective was not simply to take advantage of the sorptive capacity of the biomass but to also maintain, for as long as possible, a level of biological activity for reactor one, where sorption occurs, so that a suitable VFA production level was sustained, in keeping with normal two-phase ASBR unit operations. In addition, the maintenance of a viable anaerobic biomass in reactor one would ensure enhanced reduction of the toxic Cr(VI) to the less toxic Cr(III) species.

The cheese whey biomass demonstrated a fairly high capacity to remove Cr(VI) from solution through biosorption when compared to non-viable systems which can take advantage of optimum pH conditions to promote sorption. A q_{max} value based on the Langmuir model had a value of 5.26 g Cr(VI)/kg wet weight, which corresponds to a value of 4.74 g Cr(VI)/L settled volume or 88 g Cr(VI)/kg dry weight. The sorptive capacity of a variety of non-viable biosorbents listed in Table 2-2 of this work gave q_{max} values between 5.7 and 294 g Cr(VI)/kg dry weight

with an average of about 95 g Cr(VI)/kg dry weight. Thus, the sorptive capacity of the cheese whey biomass was similar to that obtained for non-viable biosorbents in general. However, when compared to aerobic viable biomass (activated sludge), the cheese whey biomass exhibited a much greater ability to remove Cr(VI) from solution. The sorptive capacity of viable activated sludge for Cr(III) is about 6.7 times than for Cr(VI), whereas the cheese whey biomass exhibited about a 1.8-fold enhancement for Cr(VI) when compared to Cr(III) removal for activated sludge (section 4.1.4.2). Thus, the sorptive capacity of the anaerobic biomass for Cr(VI) is about 12 times greater than that for activated sludge. From a biosorption and a Cr(VI) reduction perspective, viable cheese whey biomass presents an attractive alternative as a detoxification unit operation for industrial wastewater containing Cr(VI).

The batch test study developed an initial toxicity index based on dose rather than concentration in order to correlate toxicity with sorption capacity. The relative COD removal efficiency for increasing Cr(VI) exposure levels was used to develop an ID50 value of 283 mg Cr(VI)/L phase I optimized settled biomass. The COD removal efficiency was considered a useful parameter to investigate overall toxicity with respect to both acidogenic and methanogenic populations working in tandem. This ID50 value in conjunction with q_{max} was used to develop a preliminary model to assess the next phase of the project which was to be an extended laboratory scale ASBR experiment involving a stepwise increase in accumulated chromium in reactor one. This approach is considered an extremely

straightforward and simple one to investigate or screen the potential use of viable biomass in the two-phase ASBR approach for wastewater decontamination. The measurement of COD is readily accomplished when compared with the determination of methane production, and the results of this study indicated the usefulness of a toxicity index based on COD data as a predictive tool. Based on this approach the estimated number of one liter addition cycles of 25 mg Cr(VI)/L was determined to be between 11 and 12.

However, others have found using Cr(III) that the anaerobic digestion process exhibits a more than 2-fold sensitivity when Cr(III) is added as a one time "shock" injection when compared to a stepwise low concentration injection protocol (section 4.5). In this study an almost 3-fold increase in sensitivity to Cr(VI) was found for shock or pulsed loading of Cr(VI) when compared to stepwise, low level additions. Thus, at least 11 to 12 liters of wastewater containing 25 mg Cr(VI)/L could be expected to be processed by one liter. Indeed the ASBR experiment yielded an ID50 value of 825 mg accumulated Cr/L settled biomass based on COD removal which amounted to the treatment of 33 liters of wastewater containing 25 mg Cr(VI)/L. Using an $ID50_{avg}$ of 757 mg accumulated Cr/L settled biomass, based on methane production, relative VFA content, and COD removal, indicated that an ID50 based on COD removal alone could be used to develop a model for scale-up purposes to a pilot study. Using a 50% removal value for COD in conjunction with an accumulated chromium dose-modified reaction rate constant, derived from the ASBR study, predicted the treatment of 39 liters of

wastewater containing 25 mg Cr(VI)/L. Again, utilizing COD removal data in a simple dose effect relationship with respect to the q_{max} value represents a facile method for developing a scale-up design equation (Chapter six).

The rationale developed for scaling up the results generated in this study was to use a simple batch test to develop a conservative model for the laboratory scale ASBR setup. The performance of the laboratory scale system in relation to accumulated chromium could then be used to develop a model allowing for scale-up to a pilot size study, or aiding in estimating the cost/performance of plant size operations. In addition, the physical performance of reactor one yielded important information that could be used in conjunction with the toxicity based scale up model. The biomass characteristics indicated about a 15% decrease in reactor one TVS over a 42 day period which was accounted for by carry over loss of VSS. This loss is not considered critical with respect to overall metabolic capacity but is important when carry over of chromium into reactor two is implicated, stressing the need to reduce TVSS in the liquid effluent of reactor one or removal prior to entry into reactor two. Such information is useful for developing plant size operations and in estimating the predicted volume of spent biomass from reactor one to be handled or treated.

It should be stated that one of the advantages of using non-viable biosorbents is their potential for reuse. However, this is possible for metallic species which in general do not undergo redox reactions. It is unlikely that sorbed chromate could

be effectively removed as chromium from a non-viable biosorbent as complexation and precipitation can occur. Thus, with respect to chromium removal, this advantage is invalidated and the use of viable biomass which can accommodate both biosorption and bioaccumulation would seem preferable.

The SSE experiment indicated that Cr(III) derived from reduced Cr(VI) was essentially equally divided into two species fractions: insoluble, sorbed Cr(III)-oxides and organo-Cr(III) complexes. Although the experiment with metabolically inhibited biomass indicated that at least 25% of the sorption-reduction of Cr(VI) was biomediated, the results of the SSE experiment indicate a high degree of reduction and incorporation of Cr(VI) into the biomass granule which is consistent with a high level of cellular uptake and biomediated reduction. The sorption study indicated that viable biomass had a greater than 5-fold ability to remove Cr(VI) when compared to non-viable biomass adjusted to hydrated volume. These results imply that biomediated reduction and removal play a much greater role in the treatment of Cr(VI)-contaminated effluent in reactor one than the metabolic inhibition study indicated.

Reactor two, where methane production and recovery will take place, operated without chromium addition and demonstrated excellent performance with respect to methane production. Average production was estimated at 0.43 L CH_4/g soluble COD removed. The average methane content of the biogas was greater than 80%. The rapid reduction in methane production observed in reactor one

under Cr(VI) loading demonstrates the importance of using a two-phase ASBR system in order to both capitalize on chromium sorption and methane production capabilities of the biomass.

It should be stated at this point that although treatment of Cr(VI)-contaminated wastewater was investigated using a two-phase ASBR system, this treatment method could also be termed a two-reactor ASBR system, since it would not seem to be necessary to maintain reactor one as a phase I system in the strict sense of a traditional anaerobic two-phase reactor system. Since it is desirable to maintain a relatively low pH environment to enhance reduction of Cr(VI) to Cr(III), this low pH condition will naturally promote the growth of phase I anaerobic bacterial populations. Yet this technique can be amendable to any two-reactor system where reduction and sorption of Cr(VI) is facilitated by viable anaerobic biomass, and the effluent free of suspended chromium, as well as having a high organic loading so as to optimize methane production in reactor two.

7.2 Contribution to Knowledge

Although others have investigated the toxicity of hexavalent chromium to anaerobic biomass and have investigated the Cr(VI)-reducing capabilities of biological systems as well as their capacity to sorb chromium, the contribution of this study, related to the detoxification of Cr(VI) contaminated wastewater, is linked to its novel use of the two-phase anaerobic sequencing batch reactor

(ASBR) as a removal and detoxification technique. The present study has made the following contributions:

- The successful application of a two-phase ASBR for the removal and detoxification of Cr(VI) contaminated wastewater.
- The demonstration of the non-leachability of Cr(III) from both dried and wet spent cheese whey biomass, underscoring its suitability for disposal.
- The elucidation of a mechanism for Cr(VI) removal by granular anaerobic biomass.
- The development of a simple mathematical model based on the toxicological equivalent of a dose ($ID50_{Cr(VI)}$), with respect to biomass granules, in conjunction with adsorption data (q_{max}) to assess or screen biomass as candidates for the two-phase ASBR detoxification process developed in this study.
- The development of a simple mathematical model based on using accumulated dose to estimate the potential amount of Cr(VI)-contaminated industrial wastewater treated for pilot or plant size operation.
- The use of a modified reaction rate constant for a theoretical first order COD removal as an alternative scale-up equation, based on COD toxicity data derived from the ASBR experiment.
- The comparison of Cr(VI) toxicity to biomass under batch and semi-batch ASBR systems.

Chapter Eight

FUTURE WORK

The results of this study have demonstrated the applicability of using a two-phase anaerobic sequencing batch reactor for the removal and detoxification of Cr(VI)-contaminated industrial wastewater. However, it is believed that further work is called for. In keeping with sound environmental engineering principles, the goal of future studies would be related to increasing the treatment capacity of reactor one as well as investigating other extenuating factors which might interfere with treatment efficiency. Brief summaries of potential future areas of investigations are given below.

The effect of organic carbon substrates: This study looked at the worst case scenario in the sense that Cr(VI) was added to reactor one with a soluble, low molecular weight substrate as the carbon source. As was demonstrated, no reduction of Cr(VI) occurred when mixed with the sucrose substrate during preparation of the simulated wastewater prior to its introduction into reactor one. Thus, the granular biomass used in this study was initially exposed to the most toxic chromium species. Although this study and others have suggested the similarity in toxicity between Cr(III) and Cr(VI) for stepwise additions to anaerobic biomass, the author believes that an investigation of the effect of pre-mixing Cr(VI) with a variety of organic carbon substrates is warranted. Especially

suspended high molecular weight organic macromolecules where reduction and complexation of chromium would occur prior to entering reactor one. This may attenuate the effective chromium exposure level so as to reduce toxicity. Also a variety of low molecular weight substrates have been found to affect the rate of Cr(VI) reduction.

Investigating the effect of iron: Ferrous iron found in the reducing environment of anaerobic reactor can reduce Cr(VI) to Cr(III) resulting in the precipitation of trivalent chromium as insoluble hydroxides (Fendrof and Li 1996). Increased ferrous iron content may result in a decrease in available Cr(VI), which would otherwise oxidize the cellular material of the biomass or be transported into the cell where further cytoplasmic damage may occur. Ferrous iron content may thus attenuate the toxicity of Cr(VI). In addition investigating the involvement of ferrous iron in the reduction of Cr(VI) in anaerobic systems may further elucidate the mechanism of chromium removal.

Effect of biomass: Although viable granular cheese whey biomass proved to be a fairly effective biosorbent, screening of other granular and non-granular viable biomass material is justified; reduced sensitivity and increased sorption capacity being the goal. In addition, the settling characteristic of the biomass is an important consideration with regard to reactor design, especially with respect to carry over of chromium into reactor two. Another consideration is the drying characteristics of the biomass which is an important consideration with respect to

final sludge disposal. In addition, the availability of the biomass should be considered since anaerobic processes have low biomass yields, and thus availability may be problematic. Future research could involve the investigation and use of more widely available biosorbent materials.

Treatment of other toxic anionic metals: Other toxic metals which form stable anionic species under oxidizing conditions such as arsenic and vanadium for example can also form insoluble precipitates under reducing conditions. Future studies could assess the performance of a two-phase ASBR system with regards to treatment and removal of these toxic anionic metallic species.

Operational parameters: An investigation of the effect of detention time, pH, temperature, and mixing in order to improve or fine tune the overall operation of the system may be a useful avenue of study.

Effect of other heavy metals: Since it is expected that toxic metals other than Cr(VI) will increase the overall toxicity of the influent and possibly reduce the sorption capacity of the biomass, investigating the potential decrease in the efficacy of reactor one when other metals are present may prove of interest.

Effect of Cr(VI)/Cr(III) ratio on treatment capacity for Cr(VI): Since it is expected that the occurrence of trivalent chromium in wastewater containing Cr(VI) will reduce the capacity of the two-phase ASBR system to treat Cr(VI),

future work might attempt to delineate this effect in order to establish acceptable Cr(VI)/Cr(III) ratios with respect to cost effective treatment of Cr(VI).

Co-treatment of Cr(VI) and organic toxicants: Although this study used simulated wastewater containing readily biodegraded sucrose, it is essential that this system be tested using industrial organic toxicants as the carbon source in reactor one. Investigating whether Cr(VI) removal and reduction, in conjunction with anaerobic biodegradation of organic toxicants is feasible could be a significant area of research in order to establish greater economic feasibility.

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