REMOVAL OF COLOUR FROM SECONDARY TREATED WHOLE MILL KRAFT EFFLUENT USING DEAD *ASPERGILLUS NIGER* AS A BIOSORBENT

by

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ABSTRACT

The pulp and paper industry is widespread in Canada and many other countries. The effluent from this industry is generally highly coloured and typically measures are not taken to prevent or remove this colour due to prohibitory cost and, presently, lack of universal regulation. However, this does not preclude coloured effluents from impacting the public and the environment.

Treatment of pulp mill effluent colour has been approached using a number of different treatment technologies, including lime, membrane filtration, oxidants and adsorption. Unfortunately, biological treatment systems commonly employed to treat pulp and paper effluents are not effective in treatment of colour. However, biological treatment using live fungal biomass, generally white rot fungi, has proven to be effective at pulp mill effluent colour removal. Additionally, biosorption treatment using any biological matter in live or dead form, has been successful in the removal of metals, textile dyes and humic acids from water. Nonetheless, no research has been reported on the use of dead fungal biomass on pulp mill effluent colour. As such, the present study researches the use of dead *Aspergillus niger* biomass for the treatment of pulp mill effluent colour.

Using a batch test system approach, the present research addressed effluent characterization, pretreatment, effluent pH, biomass washing, mixing, biological inhibition, kinetic rate, isotherm, temperature, molecular weight fraction removed and practical application studies. From these studies it has been determined that autoclave-only pretreatment and initial effluent pH of 4 and 8 provided optimum colour removal. Under biologically inhibited conditions, maximum removal occurred in the first hour of biomass contact with the effluent and the kinetic models by Lagergren (1898) and Ho et al. (1996) roughly described the kinetic rate. The maximum colour removal was over 900 TCU, with a biomass doses in the range of 13-20 g/L. The equilibrium isotherms of the study fit the BET model well, which indicated, with the support of other results of the current study, that this biosorption was predominately due to physical mechanisms. In addition, the application of the biomass in a batch activated sludge process did remove colour.

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1. INTRODUCTION

1.1 Colour

1.1.1 What is colour?

Colour is a part of our daily lives. Most of world's population observes visual colour. Even though it is common to have colour vision deficiencies, it is very rare for a person to see no colour at all (The Canadian Association of Optometrists, 2005). An object's colour is frequently used as a differentiation tool, it can give beauty to an object and colour has been found to affect a person's emotional and physical condition (Nemcsics, 1993). Defining visual colour, however, is complex. The Merriam-Webster's Dictionary defines visual colour as "the aspect of objects and light sources that may be described in terms of hue, lightness, and saturation for objects and hue, brightness, and saturation for light sources" (Merriam-Webster Inc., 2004). The Encyclopedia Britannica gives the following explanations of hue, brightness (lightness), and saturation. "Hue refers to dominant wavelengths. Brightness refers to the intensity or degree of shading. Saturation pertains to purity, or the amount of white light mixed with a hue." (2005 Encyclopedia Britannica Inc., 2005). These definitions and explanations clarify what you see or how to qualitatively describe visual colour, however, they do not explain what colour is.

Matter appears coloured when visible wavelengths are absorbed. For example, an object that looks red actually is absorbing all of the wavelengths in hues other than red, such as green and blue, and is reflecting the wavelengths that are red in hue. As such, the observation of colour requires electromagnetic wavelengths, in the visible region, approximately between 380 -750 nanometers (nm), to reach the observer. In Illustration 1, the electromagnetic spectrum and the visible light spectrum are displayed. As seen in Illustration 1, red hue wavelengths are the longest visible wavelengths (lower energy) and blue hue wavelengths are the shortest visible wavelengths (higher energy). Therefore, in order for water to appear coloured, matter in the water must absorb electromagnetic wavelengths in the visible range (Kuppers, 1973).

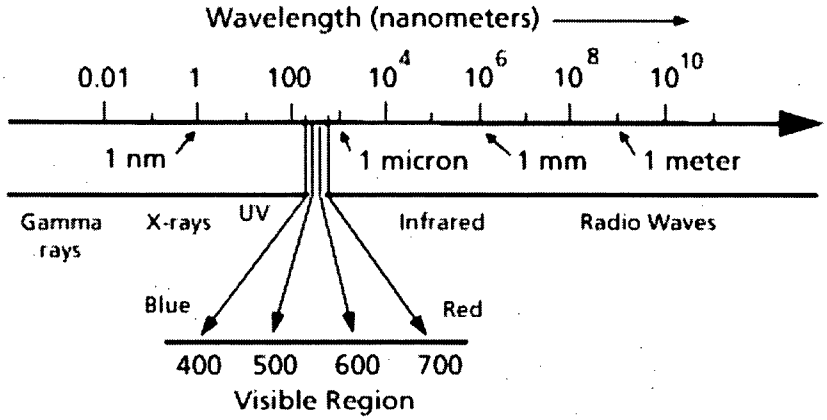

Illustration 1. Electromagnetic spectrum

Source: (ACEPT W3 Group, Department of Physics and Astronomy, Arizona State University, 1999).

Health Canada describes colour in drinking water to be the result of a number of circumstances, "... absorption of certain wavelengths of normal "white" light by dissolved or colloidally dispersed substances, by fluorescence in the visible wavelength region from substances that absorb "white" or ultraviolet light, by the presence of coloured suspended solids, and by the preferential scattering of short wavelengths of light by the smallest suspended particles." (Health Canada, 1995). This definition focuses heavily on the particles in the water. Particles can either themselves be chromophores or be attached to chromophores. Chromophore, a word originating from the Greek for "colour bringer", is a chemical group or arrangement that imparts colour (Nassau et al., 1998).

1.1.2 Origins of chromophores in water

There are multiple possible origins of colour in water bodies. All are a result of chromophores absorbing certain visible wavelengths. Generally, chromophoric molecules either contain metals or are organic substances (Health Canada, 1995).

There are many sources of naturally-occurring chromophores in water. For example, colour-imparting metals such as iron, manganese, and copper can be introduced into water from contact with geological formations, such as limestone, and colour-imparting organics can be derived from the degradation of organic matter in the environment, such as natural vegetation in soil runoff (Health Canada, 1995; Health and Welfare Canada, 1992). In addition, sources that do not occur naturally, such as industrial discharges, can also introduce colour into water bodies. The

most common coloured water-producing industries are the pulp and paper and textile industries (Health Canada, 1995). The colour imparting substances in pulp and paper mill effluents are the focus of this study.

1.2 Pulp Mill Effluents and Colour

In Canada, the pulp and paper industry extends throughout the country. In 2004, pulp and paper sales revenues were over $21 billion and production of paper and pulp products reached 30,345 thousand tonnes (Pulp and Paper Products Council, 2006). Highly coloured effluents are produced from some pulping and bleaching processes, and it is important to understand the basics of these processes.

Although nearly all pulp mills employ treatment systems, the more commonly used biological treatment systems do not remove colour effectively since effluent colour is predominately recalcitrant to biodegradation (Kemeny and Banerjee, 1997). Therefore, the treated effluent from most mills is highly coloured. There are a number of proven colour removal technologies, however, it is uncommon to see these implemented unless required by regulation due to their prohibitory cost (Springer, 1986).

1.2.1 Why remove pulp mill effluent colour?

One of the most dominant issues regarding colour in pulp mill effluents are the aesthetic impacts on the receiving water bodies. Discharges that are made to receiving water bodies, particularly at low dilution ratios, result in the receiving water appearing noticeably coloured. This detracts from visual appeal and recreational value. In addition perception set, i.e. "seeing what is expected to be seen", can impact public opinion about mill effluents (Smith et al., 1995). For example, the general feeling towards pulp mills is that they are large sources of pollution, therefore, the highly coloured water can trigger feelings that discharges are more toxic than in reality. In addition, even though regulations are in place regarding biochemical oxygen demand (BOD), suspended solids and other compounds relating to toxicity, the predominant treatment technique, activated sludge-type technology, does not remove colour effectively. This may give the impression to the unknowing eye that the effluent has not been treated well enough.

In addition, in situations where there are downstream water treatment systems, particularly for drinking water, colour intensifies the treatment required and thus displaces responsibility of treatment to the public water utility owner, typically a municipal government, instead of the producer of the effluent (Springer, 1986). In these scenarios, coloured water can pose aesthetic as well as other contamination problems.

Pulp mill effluent colour can affect aquatic systems. A very important issue is light reduction, which can affect the aquatic environment in a variety of ways. The most apparent impact of light reduction is on primary species production. There is a defined relationship between light intensity and algae growth. This not only impacts the primary species but also ripples through the entire food chain to large aquatic life forms such as fish (Rush and Shannon, 1976). Strickland noted that light is a grazing stimulus for zooplankton and is used by fish and other aquatic animals to find food (Strickland, 1958). It has also been suggested that trout avoid habitat with high suspended solids and coloured wastewater. As well, photodegradation is a naturally-occurring process that can break down undesirable compounds, such as pesticides. This process is beneficial to the environment when the by-products are not more toxic than the parent compound (Rush and Shannon, 1976). As such, light reduction is disadvantageous to this process.

Generally, studies have shown that the colour-imparting substances in effluents have no toxic effects. However, there have been a few studies that have isolated toxic substances in pulp mill effluents that are chromophores (Betts et al., 1971; Das et al., 1969). That being said, these findings have not resulted in changes to the overall consensus that colour is not toxic, since it is not consistent for general effluent types and removal of the toxic compounds can be accomplished without colour being removed (Rush and Shannon, 1976).

Nonetheless, colour-imparting substances can be toxic indirectly. Typically colour-imparting substances in the pulp mill effluent are organic compounds that have the capability to form complexes with metals (Rush and Shannon, 1976). These complexes can have impacts in two ways:

1. they can remove metals from the water body that organisms use for normal metabolism, and alternatively,

2. they can have direct inhibitory effects on some lower level organisms in the food chain (Springer, 1986).

In addition, lignin-derived colour can be an indicator for the presence of potentially inhibiting compounds and may even directly inhibit lower food chain organisms. Lastly, it has been stated that long term BOD, in the range of 20-100 days, can be exerted by colour bodies (Springer, 1986).

1.2.2 Regulation of pulp mill effluent colour

In Canada, both provincial and federal governments are involved with regulating pulp mill effluent discharges. The Canadian government has set discharge quality and monitoring requirements with the *Pulp and Paper Effluent Regulations*. These regulations under the *Fisheries Act* address BOD, total suspended solids (TSS), and toxicity (Government of Canada, Ministry of Fisheries and Oceans, 1992). Furthermore, the provinces give permission for mills to discharge under their various environmental protection acts. In some cases, the provinces have specific regulations regarding pulp mill effluent discharges, such as in British Columbia, *Pulp Mill and Pulp and Paper Mill Liquid Effluent Control Regulations*, and Ontario, *Effluent Monitoring and Effluent Limits – Pulp and Paper Sector* (Government of British Columbia, 1990; Government of Ontario, 1993). Provincial effluent quality requirements are generally equal to or more stringent than the federal *Pulp and Paper Effluent Regulations.*

Although colour limitations are not prescribed by the *Pulp and Paper Effluent Regulations,* numerous mills, particularly those that discharge to rivers or other water bodies with low dilution ratios, have colour requirements. Permits issued by the Province of British Columbia that do not require specified colour limits state:

> "Should colour, attributable to the effluent, become objectionable in the receiving environment, the permittee shall implement measures to remove colour forming constituents from the effluent." (Government of British Columbia, Ministry of Water, Land and Air Protection, 2005).

Alberta Environment has taken a more forward approach with color limits and is now imposing colour limits. In the report, *Technology Based Standards for Pulp and Paper Wastewater Releases, 2005*, the following was stated.

> "Based on a review of the current performance values for Alberta mills, other Canadian mills, and top performing mills in the U.S. and to allow sufficient flexibility for plant operations during shutdown, start-up and upset conditions, Alberta's standards for colour are a monthly average of 50 kg/ADt (air dried tonne) and a daily maximum of 100 kg/ADt for both new and existing mills. The values may be applied at approval renewal for existing pulp and paper mills, and applied directly to any new mills." (Alberta Environment, 2005).

The United States has a similar regulatory framework to that of Canada whereby the federal Environmental Protection Agency sets nation-wide standards. Individual states, however, have the ability to impose stricter effluent quality requirements (United States Environmental Protection Agency, 2002). Also, like Canada, the United States Environmental Protection Agency (US EPA) does not have general colour requirements. France does have a set limit for colour at 100 mg Pt-Co/L (milligram Platinum Cobalt per litre) for any effluent released into watercourses. Italian legislation for industrial effluents requires colour to be non-visible when dilution is 1:20, and similarly, Spain requires non-visibility at set dilution ratios (TAPPI, 1998).

2. BACKGROUND

2.1 Colour

2.1.1 Color measurement of liquid

Measurement of colour in water is typically accomplished in one of two ways, by visual comparison or through values obtained by spectrophotometric methods. Table 1 details the colour measurement methods.

Table 1. Standard methods for colour measurement

Test	Method	Standards	Use	Expression of Results
Nessler Tubes – Standard Method 2120B	Visual comparison between tubes of the sample and incremental doses of platinum cobalt	Platinum-cobalt 1 mg/L equals 1 CU, distilled water blank	Naturally coloured water, water in a hue of platinum-cobalt	Colour units (CU), true (TCU) or apparent (ACU)
Colour Wheel - Standard Method 2120B	Visual comparison with vials of sample and tinted glass covered distilled water	Tinted glass corresponding to doses of platinum-cobalt		
Spectrophotometric Method – Standard Method 2120C	Spectrophotometric measurement of transmittance at 10-30 specified wavelengths, filtered sample	Distilled water blank, percent transmittance measured	Water of any colour, particularly industrial effluents	Dominant wavelength, hue, luminance, and purity
Tristimulus Filter Method - Standard Method 2120D	Special tristimulus filters and transmittance measured	Distilled water blank, percent transmittance measured		
ADMI Tristimulus Filter Method - Standard Method 2120 E	Extension of 2120D, calculation different, use standards	Platinum cobalt standards		

(American Public Health Association et. al., 1998)

For the visual comparison method, there are two types of colour measurement, true and apparent colour. Apparent colour is measured with the liquid "as-is", whereas true colour is measured

after filtration or centrifugation. The rationale for this distinction is detailed in Section 2.1.2 regarding interference of turbidity in colour measurement.

2.1.2 Parameters affecting colour measurement

In the determination of colour, two parameters can alter measurement; turbidity and pH.

Turbidity is the only parameter that interferes with colour measurement. The presence of turbidity will interfere with colour readings since turbidity scatters light, thereby altering absorbance. Turbidity interference is an issue for both visual and spectrophotometric measurement. It is due to this interference that there are two versions of colour measurement, true colour and apparent colour (American Public Health Association et. al., 1998).

The other important parameter in colour measurement is pH. Although pH does not interfere with measurement, it greatly changes the intensity of colour. As the pH of a solution changes, the chemical make up of the solution changes. Chemical structures are altered by the abundance or lack of hydrogen ions in the solution. This affects the formation of molecules or compounds that were not previously in the solution. These changes can, in turn, alter the colour of the solution. Therefore, when measuring colour, pH should be altered to a standard value. In Standard Methods a pH value of 7.6 is standard (American Public Health Association, et. al., 1998).

2.2 Pulp Mills and Their Effluents

2.2.1 Pulping

In the creation of paper products, pulp is the fundamental raw material. Wood pulping processes de-fiber and de-knot wood chips in order to obtain the fibrous cellulose material. Pulping can be accomplished by mechanical and/or chemical means. Table 2 describes mechanical, semi-chemical, and chemical pulping.

Table 2. Pulping processes

Type	Principle	Predominate Process	Notes
Mechanical	Abrasion and friction, fibers fragmented.	Thermo-Mechanical Pulping, chips are steamed and then ground by refiners.	Softwoods, high pulp yields, poor colour impermanence and fiber strength.
Semi-chemical	Chips are "cooked" then mechanically separated.	Neutral Sulfite Semi-chemical (NSSC) process.	Mainly hardwoods, high pulp yields.
Chemical	Chemicals "cook" chips, meaning cellulose fibers are released or separated from other wood components particularly lignin.	Kraft process, "cooks" chips in a solution of sodium hydroxide and sodium sulfate to dissolve lignin.	Low raw wood quality requirements, short cooking times, well researched liquor recovery including by-product recovery, and excellent pulp strength. However, odour, low yield and highly coloured pulp are problematic.

(Fengel and Wegner, 1984; Mechanical Pulping Technical Committee, Pulp and Paper Technical Association of Canada; United States Environmental Protection Agency, 2002)

2.2.2 Bleaching

After the pulping process, regardless of method, the pulp has colour. In a large number of cases, white paper products are desired. Therefore, bleaching is a critical step in the pulp and paper process and most problematic in terms of introducing colour to effluents. Bleaching can be performed using lignin-removing or lignin-preserving bleaching procedures and these are selected based on the pulping technique utilized and requirements for the end use of the product (Sjostrom, 1981).

Kraft pulping is almost always followed by lignin-removing bleaching. Lignin-removing bleaching processes involve oxidization and alkaline extraction of lignin compounds. Oxidization breaks down lignin into water- and alkaline- soluble degradation products, and in some cases brightens the pulp (Sjostrom, 1981). The purpose of alkaline extraction is the "removal of lignin degradation products in combination with a neutralization of acidic components formed during oxidation" (Fengel and Wegner, 1984). Table 3 lists commonly used bleaching stages and the corresponding symbols widely used to indicate the processes.

Table 3. Common bleaching stages

Stage	Symbol	Use
Chlorination	C	Typically first stage (pre-bleaching), breaks lignin bonds using Cl_2
Alkaline Extraction	E	Dissolve reaction products using NaOH
Chlorine Dioxide	D	Delignifies and brightens pulp using ClO_2
Ozone	Z	Delignifies and brightens pulp using O_3
Oxygen	O	Delignifies using O_2, but not selective to lignin
Peroxide	P	Mainly latter stages, brightens using H_2O_2

(Fengel and Wegner, 1984; TAPPI, 1997)

Bleaching sequences are listed using these symbols in the sequence order. Additionally, subscribed letters indicate a stage that is enhanced with those processes. For example, the bleaching sequence CDEopCD indicates the following stages chlorine, chlorine dioxide, alkaline extraction enhanced with oxygen and hydrogen peroxide, chlorine, and chlorine dioxide. In lignin-removing bleaching processes, at least three of these stages and in some cases up to seven stages are applied.

2.2.3 Effluent management

Mills have two potential effluent management approaches: 1. internal process modification to reduce contaminants and effluent quantities, or 2. external treatment processes that are additional to the plant production processes (Springer, 1986). Typically, a combination of these methods is used.

Process modification can be aimed at the reduction of effluent contaminants, such as the use of elemental chlorine-free bleaching, or at reduced water consumption, such as the optimization of production water use (Springer, 1986; TAPPI, 1998). Some mills types have even been able to achieve zero effluent discharge (Forrest, 1992). Process modification improvements can also decrease the amount of colour in effluents. Reduction in colour can be achieved through bleaching sequence modifications and reuse and recycling of high colour streams (Rush and Shannon, 1976).

There are a variety of external treatments for effluent. Physical and physical/chemical treatments are the principal methods for removal of solids. Pulp mills employ physical and

physical/chemical effluent treatment methods such as coagulation/flocculation followed by settling or flotation, sand filtration or ultrafiltration (TAPPI, 1998). Biological treatments, primarily used for treatment of dissolved or colloidal matter, include aerated stabilization basins, activated sludge treatment systems and anaerobic treatment systems and are widely used in the pulp industry (Springer, 1986).

2.2.4 Origins of colour in effluents

The effluent streams in a mill that are the most coloured come from pulp cooking and bleaching processes (Springer, 1986). The effluent from the pulp cooking, suitably referred to as "black liquor", contains "degraded thiolignins, degraded carbohydrates, and small amounts of fatty and resin acids and other extraneous material." (Dugal et al., 1974). The bleach plant effluent also contains lignins, as a result of the further delignification and oxidation of the pulp, as well as small amounts of other compounds. It has been noted that the bleach plant effluent can account for as much as two-thirds of the colour load but only one-third of the effluent volume (Springer, 1986).

The material responsible for imparting colour to these effluents has not been clearly defined. Different theories have been developed regarding its source, such as colour compounds being carbohydrate-derived (Ziobro, 1990). However, the general consensus amongst experts credits lignin and lignin-derived compounds for imparting colour due to lignin's high degree of conjugation (Springer, 1986). For example, a study performed by Suckling and Pasco in 2001 found that carbohydrate-derived chromophores could only contribute slightly to the absorbance of kraft spent liquor. Also, lignin-removing bleaching procedures contribute the most colour to effluents in comparison to pulping and other bleaching processes (Springer, 1986).

In addition to these pulping and bleaching colour sources, a recent study by Milestone et al. (2004) revealed that certain conventional treatment systems can increase colour by an average of 20-40% and up to 50%. The study showed this colour increase occurred in aerated stabilization basins (ASB), but not activated sludge systems, and only in regions of negative or low redox conditions.

2.2.5 Lignin

Generally speaking, the main macromolecular cell wall components in wood are cellulose, polyoses (hemi-cellulose) and lignin (Fengel and Wegner, 1984). Lignin is a polymeric organic substance that gives strength to wood. It is the last macromolecular substance formed in the plant cell wall and, therefore, is infused within the other cell wall components, including the desired cellulose fibers. The removal of lignin is important in pulping not only because lignin acts like "glue" holding the cellulose fibers together but it also imparts colour to the pulp. Therefore, during the pulping process as much lignin is removed as possible without compromising pulp quality. Softwoods contain more lignin than hardwoods. As well, lignin and most lignin-derivatives are high molecular weight molecules (Sjostrom, 1981).

The lignin-based colour theory is supported by characteristics of the pulp mill effluents. Lignin shows predominate absorbance of the short wavelengths in ultraviolet (UV) spectroscopy, which corresponds to the brown-red hue pulp mill effluents (Springer, 1986). In addition, the colour in pulp mill effluents is largely recalcitrant which reflects lignin's high resistance to microbiological degradation (Dugal et al., 1974).

2.3 Current Colour Removal Technologies

Although a description of every technology is not possible, the most feasible and well studied treatment techniques are described in this section.

2.3.1 Chemical treatment using aluminum and ferric salts

A large number of studies have shown aluminum and ferric salts to be highly effective at removal of colour, with efficiencies ranging from 85-95% (Rush and Shannon, 1976). Aluminum and ferric salts are effective because they act as both a coagulant and a precipitant (Tchobanoglous et al., 2003). In chemical coagulation, colloidal matter is destabilized in order for flocculation to occur. Flocculation is the increase of particle size as a result of particle collisions. Destabilization arises when the stronger repelling electrical charge forces of a colloid no longer prevent attraction to other particles. Alternatively, chemical precipitants alter the state of dissolved and suspended matter in order to alter matter formerly unable to settle, to a form that is able to settle. Although aluminum and ferric salts are very effective for colour removal, there are a number of drawbacks to these removal techniques, in which the main issues are: "1.

need for precise pH and zeta potential monitoring and control (this makes process automation difficult), and 2. problems encountered in sludge handling." (Rush and Shannon, 1976).

2.3.2 Chemical treatment using lime

The National Council of the Pulp and Paper Industry for Air and Stream Improvement pioneered effluent colour removal technologies using lime (Dugal et al., 1976). Use of lime was an attractive option since it was already being used at most mills in the recovery process, and therefore lime colour treatment could be easily integrated into the mill processes (Rush and Shannon, 1976). Lime treatment is a precipitation process in which removal is largely dependant on the formation of insoluble calcium-organic salts.

Massive lime treatment, modified lime treatment and minimum lime treatments have been put into practice in operating mills and studied extensively (Oswalt and Land, 1973; Rush and Shannon, 1976; Spruill, 1973) Massive lime treatment uses lime doses of about 20,000 mg/L and is capable of overall mill effluent colour removal of about 72%. The textbook "Industrial Environmental Control: Pulp and Paper Industry" explains massive lime treatment as follows: (note that green liquor is a liquid formed during the sulfate recovery process and white liquor is the refortified green liquor used for pulping) (Lavigne, 1993)

> "In the massive lime process, the mill's total lime supply is slaked and reacted with a small-volume, highly colored stream, usually the bleach plant caustic effluent, at a lime concentration of 20,000 mg/L. The lime is settled, dewatered, and used for causticizing green liquor. The color bodies dissolve in the white liquor and eventually find their way into the recovery furnace." (Springer, 1986).

Colour removal using lime has drawbacks. Three of the main issues are:

1. inability to treat the entire effluent stream,
2. dilution of the cooked liquor requires increased treatment and recovery capacities, and
3. foaming in the clarifier and of the cooked liquor (Rush and Shannon, 1976, Springer, 1986).

Modifications to lime treatment were developed, aimed to minimize the problems of the massive lime process. The modified lime treatment replaced a large portion of the colour effluent slaked lime with lime mud from the recarbonation clarifier. This reduced foaming issues (Rush and

Shannon, 1976). Minimum lime treatment used lime doses closer to the stoichiometric quantities, which solved problems that developed in the massive lime process but in turn resulted in other issues such as colour reversion (Springer, 1986).

2.3.3 Other chemicals used to enhance settling

Beyond the conventional coagulants, there have been experimental trials using synthetic coagulants such as hexamethylene diamine epichlorohydrin polycondensate (HE) and polyethyleneimine (PE) and natural coagulants such as chitosan, that have been successful in the removal of colour (Ganjidoust et al., 1997). Also, conventional chemical settling aids such as lime and ferric chloride have been used in combination to enhance removal already seen with these chemicals independently (Dugal et al., 1976). There are many possible coagulants and chemical variations that have been found to remove colour, all achieving relatively similar results. It can, therefore, be concluded that coagulant and precipitant technologies can be effective for the removal of colour, however cost and practicality of use are limiting factors.

2.3.4 Chemical oxidation

Chemical oxidization is a treatment process wherein colour molecules are destroyed rather than removed. Ozone, hydrogen peroxide, chlorine dioxide, chlorine and hypochlorite, peracetic acid and Caro's acid have all been studied for the treatment of coloured pulp effluents (Springer, 1986). However, cost is a major restrictive issue with the use of oxidants. In addition, less costly oxidants such as chlorine and hypochlorite have other unwanted effects. Chlorination with chlorine or hypochlorite forms unwanted halogenated organic by-products. (Hao et al., 2000) Oxidants have also been employed as a pretreatment to biological treatment, as they can break down recalcitrant organics.

Ozonation is effective both as a treatment and pretreatment method. Bauman and Lutz found that the application of 30-40 part per million (ppm) of ozone on secondary effluent reduced colour by 60-70 percent (%), however, it did result in an increase of BOD (Bauman and Lutz, 1974). Ozone as a pretreatment has also proven to be effective in the breakdown of recalcitrant colour compounds (Bijan and Mohseni, 2004). The main issue with ozone is its acquisition, in that on-site generation is generally required which affects cost.

2.3.5 Advanced oxidation processes

Advanced oxidation processes are based on the formation of extremely reactive radical species, namely hydroxyl radicals (Hao et al., 2000). Utilization of ozone, ozone and hydrogen peroxide, Fenton's reagent (H_2O_2 and Fe II), UV/peroxide/ozone, and UV and titanium dioxide are all examples of known combinations that will form hydroxyl radicals (Hao et al., 2000; Sevimli, 2005). In a comparison of homogeneous and heterogeneous ozone applications by Mansilla et al., 1997, ozone used in conjunction with titanium oxide or zinc oxide achieved nearly 40% removal of colour in the first minute in the bleach process effluent compared to just ozone, which required 15 minutes to achieve 30% removal. Sevimli (2005) performed Fenton's oxidation studies in which 95% of colour was removed from biological treated pulp and paper effluents.

2.3.6 Biological treatment

Biological treatment is an important category in the treatment of pulp mill effluent colour. As mentioned in Section 1.2.3, conventional biological treatment technologies, including activated sludge and aerated stabilization basins, are the most widely used effluent treatment processes in the pulp and paper industry, yet they are not effective in the removal of colour. Process enhancements, such as oxidation pretreatment, have the ability to increase the biological removal of colour through breakdown of the recalcitrant colour bodies into readily degradable matter.

Non-conventional biological treatments using white rot fungus have proven to be successful. A full discussion of these treatments can be found in Section 2.4.

2.3.7 Membrane treatment

Membrane treatment, primarily ultrafiltration, has also been successful in the removal of colour. Membrane filtration is the separation of particulate, colloidal and dissolved constituents down to the fractions of a micron size range from a liquid (Tchobanoglous et al., 2003). In membrane treatment, the semi-permeable membrane produces two liquid streams; the permeate, or the treated stream, and the concentrate, or the liquid that does not pass through the membrane. The driving forces used for treatment, either hydrostatic pressure difference as in reverse osmosis, or dialysis type membranes that use concentration gradients and electrical potential, can distinguish membranes types. Membrane systems that use hydrostatic pressure will be the focus of this

discussion. Table 4 lists these membranes and the typical operating ranges and mechanisms for removal.

Table 4. Membrane filters characteristics

Membrane Type	General Separation Mechanism	Minimum particle size removed, μm
Microfiltration	Sieve	0.08 – 2.0
Ultrafiltration		0.005 – 0.2
Nanofiltration	Sieve + solution/diffusion + exclusion	0.001 – 0.01
Reverse Osmosis		0.0001 – 0.001

(Tchobanoglous et al., 2003)

A range of membrane processes from ultrafiltration through reverse osmosis has been studied for treatment of pulp mill colour. Nevertheless, ultrafiltration has been the membrane process of choice. In 1973, Freemont et al. (1973) did a study for the US EPA using ultrafiltration and found colour removal efficiencies on kraft pulp bleachery effluents to be in the range of 90-97%. Ultrafiltration and nanofiltration membranes achieved 92% and 100% colour removal, respectively, on first stage alkaline extraction effluents (Rosa & Depinho, 1995).

The problems associated with membrane filtration of pulp effluents are concentrate disposal, membrane fouling, and cost. Additionally, membrane treatment in pulp mills is generally seen as a polishing step or is relegated for use on specific effluent streams (Springer, 1986).

2.3.8 Resin separation and ion exchange processes

Ion exchange is a term that has been loosely used for processes that include true ion exchange, a combination of ion exchange and adsorption and in some cases only adsorption. What appears to be common with this group of technologies is the use of resins that require regeneration. The fundamentals of adsorption will be explained in Section 2.4.

Ion exchange processes displace ions from a resin, an insoluble exchange material, with ions in a solution. There are many categories of resin types; strong-acid cation, weak-acid cation, strong-base anion, weak-base anion, and heavy-metal selective chelating resin (Tchobanoglous et al., 2003). Generally weak-base anionic resins are most effective on pulp mill effluents. It is important to note that the higher capacity the resin, the shorter lifespan it will have. Generally, the lifespan of resins is short, up to five years (Springer, 1986).

Under the title of ion exchange, two notable processes were developed that have been found to be successful for the treatment of coloured effluents; the Rohm-Haas process and the Uddeholm-Kamyr process. The Rohn-Hass process is an adsorption-based process, since the resin used, Amberlite XAD-8, a highly cross-linked hydrophilic, porous polymer, contains no ion exchange groups (Rock et al., 1974). Nonetheless, this process was able to remove 70-95% of colour from a combined alkaline extraction and chlorine effluent. The Uddeholm-Kamyr process works on the principles of both ion exchange and adsorption using a weak-base anionic resin (Rush and Shannon, 1976). This approach has been shown to remove 80-95% of colour from caustic extraction effluent.

2.3.9 Activated carbon adsorption

Activated carbon is charred organic material that is heated to just below 700°C under low oxygen conditions to prevent combustion, and then activated by exposure to oxidizing gases, such as steam or CO_2, again at high temperatures (Tchobanoglous et al., 2003). This creates the all-important porous structure of activated carbon. Activated carbon is used in two forms, powdered or granular, and is referred to as powdered activated carbon (PAC) or granular activated carbon (GAC). GAC is used in a filter column and, therefore, generally requires its own apparatus. On the other hand, PAC can be added virtually anywhere. PAC has been added to the head end of the aeration tanks in activated sludge systems (United States Naval Facilities Engineering Service Center, 2002). Activated carbon has a relatively short lifespan but can be regenerated.

Activated carbon alone has been found to remove over 50% of colour in pulp effluents (Jacksonmoss et al., 1992). Interestingly, low molecular weight particles are more readily adsorbed by activated carbon and this makes addition of carbon an excellent polishing step (Rush and Shannon, 1976). Most colour removal technologies, such as lime, remove the larger molecular weight portions. The patented ISEP system "allows continuous operation of all processing steps in any staged sorption process without resorting to slurried transport of the sorbent material or use of batch sequencing techniques." (Springer, 1986). ISEP uses three steps; acidification, settling and activated carbon adsorption and can achieve 90-95% colour removal from final effluent (Springer, 1986).

2.4 Mechanisms of Adsorption

2.4.1 Basics of adsorption

Table 5 provides definitions of terms important for understanding adsorption.

Table 5. Definitions of adsorption terms

Term	Definition
Sorption	A process where contaminants move, or concentrate, from one phase to another.
Adsorption	Process whereby ions or molecules in one phase have a tendency to accumulate on the surface of another phase.
Absorption	Partitioning of specific constituent from one phase to another.
Adsorbate	A substance that is being removed from the liquid phase at the interface.
Adsorbent	The solid, liquid or gas phase on which the adsorbate accumulates.
Bulk liquid	The liquid phase where the constituent concentration is uniform due to advection and dispersion.
Stagnant liquid film	A film of liquid next to the adsorbent where the constituent concentration is decreasing from the bulk liquid.

(Tchobanoglous et al., 2003; Sawyer et al., 2003)

Adsorption, like absorption, falls under the general category of sorption, however, as described in Table 5, these processes are quite different. Adsorption occurs in four general steps:

1. bulk solution transport,
2. film diffusion transport,
3. pore transport, in the case of a porous absorbent, and
4. adsorption (Tchobanoglous et al., 2003).

Bulk solution transport describes the movement of the adsorbate through the bulk liquid to the stagnant liquid film (Tchobanoglous et al., 2003). Next, the adsorbate is transferred through the stagnant film layer by diffusion to the entrance of the pores of the adsorbent. This is called film diffusion transport. Pore transport is the movement of the adsorbate through the pores by molecular diffusion via the pore liquid and/or diffusion along the surface of the adsorbent. Lastly adsorption occurs where the adsorbate attaches to the adsorbent at an available adsorption site.

Adsorption is a result of many potential mechanisms. *Wastewater Engineering – Treatment and Reuse* by Tchobanoglous et al. (2003), lists the following adsorption forces: coulombic-unlike

charges, point charge and a dipole, dipole-dipole interactions, point charge neutral species, van der Waals forces, covalent bonding with reaction and hydrogen bonding. Generally, these forces are classified into two categories; physical and chemical adsorption (Sawyer et al., 2003). Physical adsorption is attributed to the existence of weak attractive forces, or van der Waals' forces, between molecules. Physically adsorbed particles are not fixed to a particular site on the solid surface but can move around on the surface, allowing more than one layer to form. This type of adsorption is typically quite reversible. Chemical adsorption, often entitled chemisorption, involves a strong adsorption force that generally only allows a single layer of adsorbed molecules to form. Also, molecules are not free to move along the surface and the adsorption is rarely reversible.

2.4.2 Adsorption rates

An important characteristic of an adsorbent is the rate at which adsorption equilibrium occurs between the adsorbent and the adsorbate at a specific initial concentration. Two equations have been commonly used to describe adsorption rates, the Lagergren equation and the Ho et al. equation. (Ho et al., 1996; Lagergren, 1898)

The Lagergren equation is a first order rate equation, although it is commonly referred to as a pseudo-first order rate equation "to distinguish kinetics equation based on sorption capacity of solid from concentration of solution, ..." (Ho, 2004). Equation 1 is the Lagergren equation,

$$\ln(q_e - q_t) = \ln(q_e) - Kt \qquad (1)$$

and will be used in the present study in the re-arranged form, Equation 2, as follows,

$$q_t = q_e(1 - e^{-Kt}) \qquad (2)$$

where q_e is the amount of colour removed, per mass of adsorbent (mg/g), at equilibrium, q_t is the amount of colour adsorbed, per mass of biomass (mg/g), at time t (h) and K (1/h) is the rate constant per unit time (Lagergren, 1898).

Ho et al. discovered that the Lagergren equation, although it applied very well to some situations, did not describe all situations. Therefore, they developed the following, Equation 3, of the pseudo-second order to describe a multi-component system,

$$\frac{t}{q_t} = \frac{1}{2kq_e^2} + \frac{t}{q_e} \qquad (3)$$

which was re-arranged for use in the present study as Equation 4,

$$q_t = \frac{2kq_e^2 t}{1 + 2kq_e t} \tag{4}$$

where k (g/mg·h) is the rate of sorption (Ho, 2004).

2.4.3 Adsorption isotherms

Isotherms are used to describe equilibrium between the quantity of adsorbate in solution and the quantity of adsorbate on the adsorbent at a given temperature. This equilibrium is based on properties and quantities of both the adsorbate and adsorbent. Solubility, molecular structure, molecular weight, polarity, and hydrocarbon saturation are important characteristics of the adsorbate (Tchobanoglous et al., 2003). The widely used Langmuir, Freundlich and Brunauer, Emmet and Teller (BET) isotherms are described below.

The Langmuir isotherm describes a monolayer surface adsorption on an ideal surface. The Langmuir theory is based on the kinetic concept that there is continual bombardment of molecules onto the surface and a simultaneous desorption of molecules from the surface to maintain a zero rate of accumulation at equilibrium (Do, 1998). The assumptions of this model are:

1. the surface is homogeneous, i.e. adsorption energy is consistent at all sites,
2. molecules are adsorbed at a definite, localized site, and
3. each site can only accommodate one molecule.

The Langmuir model is described by Equation 5,

$$q_e = \frac{Q^o bC}{(1 + bC)} \tag{5}$$

where q_e (mg/g) is the amount of constituent adsorbed per unit weight of adsorbent at concentration C, Q^o (mg/g) is the amount of constituent adsorbed per unit weight of adsorbent in forming a complete mono-layer, b is a constant related to energy of adsorption and C (mg/L) is the measured concentration of the constituent, in solution, at equilibrium (Sawyer et al., 2003).

The Freundlich equation is a long-used empirical equation, commonly applied to describe activated carbon adsorption. In spite of its empirical origins, this equation can be derived from the Langmuir equation by assuming that the adsorbent surface has a heterogeneity of adsorption

energies and that sites having the same adsorption energies, are grouped together (Do, 1998). Equation 6 is the Freundlich equation.

$$q_e = K_F C^{1/n} \tag{6}$$

where K_F and $1/n$ are the Freundlich constants indicating adsorption capacity of adsorbent and changes in affinity for the adsorbate with adsorption density, respectively (Sawyer et al., 2003). The Freundlich isotherm becomes linear when $n = 1$, meaning that all adsorption sites have equal affinity for the adsorbates.

Multi-layer adsorption can be described by the BET theory. BET theory makes the same assumptions as the Langmuir theory, however, more than one layer of adsorbate can form. Consequently, the BET theory applies the kinetic principles of adsorption and desorption of the Langmuir theory to every layer. Equation 7 is the BET equation

$$q_e = \frac{BCQ^o}{(C_s - C)\left[1 + (B-1)(C/C_s)\right]} \tag{7}$$

where C_s (mg/L) is the saturation concentration of constituent in solution, and B is the constant regarding energy at the surface (Sawyer, et al., 2003).

In addition, the Brunaner, Deming, Deming, Teller (BDDT) classification system typifies real world experimental results into five categories of isotherms. This classification is listed Table 6.

Table 6. BDDT isotherm classifications

Type	Standard example	Comments
I	Adsorption of oxygen on charcoal at -183°C	Langmuir isotherm type (monolayer coverage)
II	Adsorption of nitrogen on iron catalysts at -195°C	Demonstrates the BET adsorption mechanism
III	Adsorption of bromine on silica gel at 79°C, water on glass	Type where adsorption is not favourable at low pressure, hydrophobic adsorbent
IV	Adsorption of benzene on ferric oxide gel at 50°C	Similar to type II except has a finite limit
V	Adsorption of water on charcoal at 100°C	Similar to type III except has a finite limit

(Do, 1998)

Types I through III can be described by the BET theory (Do, 1998). Types IV and V do not follow the BET theory as it assumes there is a infinite number of layers of molecules and types IV and V have a finite limit.

The International Union of Pure and Applied Chemistry (IUPAC) has accepted the five types described by Brunaner, Deming, Deming, Teller described above, as well as a sixth type that details a macroporous structure where stepwise multilayer adsorption occurs (Sing et al., 1985).

2.4.4 Adsorption of heterogeneous mixtures

Compared to a pure solution, a mixture generally shows a decrease in total adsorbance for a specific compound, however, the total adsorbance capacity for the mixture may be greater than it is for that specific compound (Tchobanoglous et al., 2003). Competition for adsorption sites is dependant on the size of the molecules being adsorbed, adsorption affinities and relative concentrations. That being said, the isotherms described in Section 2.4.3 can be applied to heterogeneous mixtures.

2.5 Fungal Biosorption

2.5.1 Use of biosorption

Biosorption is a loose term used to describe the removal of contaminants from a solution by sorption on biological material (Zhou and Banks, 1991). Biosorption consists of an assortment of removal mechanisms including adsorption and precipitation, as well as, in the case of living biological matter, metabolic activity (Gadd, 1992). Any biological matter can be a biosorbent, yet, research in this field has focused on microorganisms of all classes, including algae, bacteria, fungi and yeasts. Further, the definition of biosorption includes organisms in any life state, i.e. dead or alive, any component of the organism, and any matter the organism may have produced.

The majority of work classified under the category of biosorption has been in the treatment of metals, dyes and pulp mill effluents. This work has been quite successful and thus research has been extensive, investigating a wide range of biosorbents. Several researchers have utilized fungus, treating a variety of metals, dye types and pulp mill effluent streams (Banat et al., 1996; Fu and Viraraghavan, 2001a; Garg and Modi, 1999; Ho and McKay, 2003; Kapoor and Viraraghavan, 1995; Polman and Breckenridge, 1996). Dyes, and in some cases metals, are

chromophoric substances. As well, numerous chromophores exist in the heterogeneous mixtures of pulp mill effluents. There has also been work in the area of naturally occurring chromophores like humic acids. Zhou and Banks found that filamentous fungus *Rhisopus arrhizus* was successful in the removal of colour from naturally coloured waters (Zhou and Banks, 1991).

The major reason for the popularity of research in biosorption is economics. In comparison to conventional treatment technologies biosorption has the potential to be low cost in addition to being relatively easy to implement (Banat et al., 1996).

2.5.2 Fungal biosorption

In the realm of biosorption research, there has been particular focus on the use of fungal biomass. Fungal biomass has been applied in both living and dead states, but the removal mechanisms are different, although some commonality exists.

Living fungal cells utilize metabolic activity to treat waters. There are generally two categories of live fungi used; white rot fungi and other fungi.

White rot fungi, also known as wood-rotting fungi, which fall under the category of *Basidiomycetes*, were first used to treat pulp mill effluents (Demain and Soloman, 1986; Hawker and Linton, 1979). Notably, Fukuzumi et al. (1977), and, Eaton et al. (1980), reported successes with the use of the species *Tinctoporia* species and *Phanerochaete chrysosporium,* respectively. As a result, white rot fungi have been researched extensively for the breakdown of recalcitrant compounds in pulp and paper effluents and a variety of dyes. A large number of researchers have used *Phanerochaete chrysosporium*, whereas others have selected different species of white rot fungi, for example, *Trametes versicolor, Coriolus versicolor*, and *Ceriporiopsis subverispora* (Bergbauer et al., 1991; Bilgic et al., 1997; Glenn and Gold, 1983; Mittar et al., 1992; Nagarathnamma et al., 1999; Pastigrigsby et al., 1992; Royer et al., 1991; Sundman et al., 1981). *Phanerochaete chrysosporium* is used in the registered process MYCOR® for the treatment of pulp mill effluents (Springer, 1986). White rot fungi are unique as they produce ligninolytic enzymes: laccase, manganese peroxides (MnP) and lignin peroxidase (LiP), to biodegrade recalcitrant organics such as lignin and lignin-derived compounds found in pulp mill effluents. In addition to biodegradation, white rot fungi cells are capable of facilitating adsorption to the cell wall (Fu and Viraraghavan, 2001a).

Successful removal of recalcitrant organics is also possible with other fungal types. However, since they do not have the lignin-modifying enzymes, they rely on other mechanisms for removal. Zhou and Banks observed biodegradation of humic acids with *Rhizopus arrhizus* by use of the cell's metabolic energy (Zhou and Banks, 1991). This process is thought to involve the uptake of compounds, or their degradation products by the cell, but this process occurred as a second phase of removal. The first phase of removal involved adsorption of constituents to the cell wall (Zhou and Banks, 1991). In addition to *Rhizopus arrhizus,* other fungi have been used to remove recalcitrant organic material, such as *Rhizopus oryzae* and *Aspergillus niger* (Kapoor, 1998; Nagarathnamma and Bajpai, 1999).

Some research has shown success using dead fungal biomass for removal of metals, humic acids, phenol and dyes. In these studies, removal of dead biomass was a result of adsorption, almost exclusively physical (Fu and Viraraghavan, 2000; Gallagher et al., 1997; Kapoor et al., 1999; Rao, 2001, Zhou and Banks, 1991). Many studies have attributed the majority of removal to chitin/chitosan in the cell wall of the fungus (Banks and Parkinson, 1992; Zhou and Banks, 1991). Chitin is a complex amino-polysaccharide that is found in the cell wall of many moulds, as well as other organic matter such as crab shell, and chitosan is the deacetylatized form of chitin (Hawker and Linton, 1979).

A benefit to the use of dead biomass is the employment of pretreatments. Alteration of biomass cell structure or surface charge can significantly change the biosorption ability of fungal biomass as it is usually negatively charged. A wide variety of chemical and physical modifications have been tested with varying results, depending on the characteristics of the targeted constituents (Fu and Viraraghavan, 2001b; Gallagher et al., 1997; Kapoor, 1998). For example, Gallagher et al. (1997), found alkali pretreatment using NaOH increased adsorption of humic acids as it increased anionic sites on the cell wall by exposing the chitin/chitosan complex. In addition, acid pretreatment, using acids such as HCl and H_2SO_4, have been used in attempt to neutralize or change the surface charge of the biomass, and salt pretreatment, using such salts as $NaHCO_3$ or $CaCl_2$, can also affect the surface charges (Fu and Viraraghavan, 2001b). Autoclaving increases the surface area and monolayer volume of a cell (Gallagher et al., 1997). As well, recent studies have isolated certain cell components for use as absorbents. For example, chitosan beads have been reported to be successful biosorbents (Chiou et al., 2003).

As both live and dead fungi have proven to be potential biosorbents, it is important to compare the two life states. Table 7 is a comparison of the adsorption characteristics of living and dead cells.

Table 7. Comparison of adsorption characteristics of life states

Issue		Live	Dead	Citation
Biosorption capability	Dyes	Excellent	Excellent	Fu and Viraraghavan (2001a)
	Humic acids	Excellent	Excellent	Zhou and Banks (1991)
	Metals	Excellent	Excellent	Kapoor and Viraraghavan (1995)
	Pulp mill effluents	Good/Excellent[1]	No work found.	Bilgic et al. (1997)
Operation	Additional chemicals	Must ensure readily available food source and nutrients.	Chemical elution for regeneration.	Fu and Viraraghavan (2001a)
	Configuration	Either suspended or fixed film, such as a rotating biological contactor.	Powder addition or immobilized biomass in filter column.	
	Other	Short biomass life span. Need to ensure solution to be treated in not toxic to biomass.	Used either in powdered or column forms.	

Notable, in Table 7, is the absence of research in the area of pulp mill effluents using dead biomass.

2.5.3 Factors affecting dead fungal biomass biosorption

In dead biomass studies, researchers have noted that certain constituents or physical properties of the solution affect biosorption. Table 8 lists these constituents and physical properties.

Table 8. Factors affecting biosorption

Property	Affect on biosorption	Mechanism	Citation
pH	Both increase or decrease depending on target contaminant and pH.	Charge neutralization or change.	Fu and Viraraghavan (2001b)
Metal ions	Increased humic acid uptake.	Bridging of cell wall surface charge.	Zhou and Banks (1991)
Ionic strength	Increased humic acid uptake.	Surface chemistry forming an electric double layer.	Zhou and Banks (1993)
Temperature	Physical adsorption generally decreases with increased temperature. Chemical interactions increase with temperatures.	Physical adsorption surface loading capacity increases with decreasing temperature.	International Union of Pure and Applied Chemistry (1971)

Additionally, Zhou and Banks studied the effects of biomass culture age and growth medium on adsorption performance (Zhou and Banks, 1993). They found that biomass age did affect biosorption performance, with four days being the optimum age for *Rhizopus arrhizus*. In terms of growth medium, they also found this to have an effect, with *Rhizopus arrhizus* grown in potato dextrose liquid medium being the most effective compared to other *Rhizopus arrhizus* biomasses. Both of these improvements were related to an increase in chitin/chitosan content of the cells (Zhou and Banks, 1993).

2.5.4 Aspergillus niger

A variety of dead fungal biomasses have been used as biosorbents such as *Myrothecium verrucaria*, *Rhisophus arrhizus*, and *Rhizopus oryzae*, including *Aspergillus niger* (*A. niger*), which has been used in the removal of metals, dyes and phenol (Fu and Viraraghavan, 2000; Gallagher et al., 1997; Mou et al., 1991; Rao, 2001; Zhou and Banks 1991).

A. niger is classed as *Fungi Imperfecti*, essentially a fungi that reproduces asexually (Hawker and Linton, 1979). It also falls in the sub-class *Hyphomycete*, commonly called moulds, which is a diverse group of fungi that produces conidia (a spore type). In the case of *A. niger*, conidia are grown on the end of a conidiphore, a stem-like structure that grows from the fungal mass or mycelium. Another popular fungal strain in this category is *Pencillium*. *A. niger* is characterized by its "mould-like" appearance with a white-creamy coloured mass and black conidia.

A. niger exhibits many advantageous characteristics for use as a biosorbent. First and very importantly, is its wide spread use in industry. The US EPA's *Aspergillus niger Final Risk Assessment* stated that *A. niger* is most commonly used for the production of enzymes such as aamylase, amyloglucosidase, cellulases, lactase, invertase, pectinases, and acid proteases, and the fermentation production of citric acid (United States Environmental Protection Agency, 1997). This is very important as there is the potential to use waste *A. niger* as an economical biosorbent supply source. In addition, *A. niger* is a classified under Biosafety Level 1 by the US Center for Disease Control. "Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing." (U.S. Department of Health and Human Services, 1999). Lastly, due to the effective reproductive techniques of *A. niger,* biomass growth is effortless (Hawker and Linton, 1979).

2.5 Background Conclusions

Coloured pulp mill effluent discharges can affect the public via aesthetic related issues, the aquatic environment and downstream water treatment systems. Although there has been a great deal of research regarding the prevention and treatment of coloured effluents, economical treatment solutions have not been developed. As a result, pulp mills have generally not adopted colour-removing techniques, unless required by law.

In addition, research using live fungi for removal of colour in pulp mill effluents and dead fungal biomass for removal of dyes, metals and other contaminates has been widespread. Nonetheless, based on the information collected, it appears that no research has occurred using dead fungal biomass for the removal of pulp mill effluent colour. Therefore, dead fungal biomass biosorption has potential to be an economical effluent colour removal technology for the pulp and paper industry.

3. OBJECTIVES

The overall objective of this study was to determine if inactivated fungal biomass of *A.niger* effectively removes colour from pulp mill effluent. This overall objective was achieved through three minor objectives.

1. To determine if dead fungal biomass *A. niger* would remove colour from treated whole mill effluent in a laboratory setting.
2. To optimize and understand the mechanisms for removal of colour from treated whole mill effluent in a laboratory setting using dead fungal biomass *A. niger*.
3. To determine if dead fungal biomass *A. niger* would remove colour from primary clarified effluent in an activated sludge process.

4. METHODOLOGIES

The current research consisted of three main phases:

1. effluent characterization and biomass production,
2. batch biosorption study, and
3. practical application study.

4.1 General

4.1.1 Pulp mill effluents

Two effluents were studied in this research. The first effluent was collected on April 20, 2005, from the former Western Pulp Partnership Ltd. pulp mill near Squamish, British Columbia. The sample was whole mill effluent after secondary treatment. This mill produced a northern bleached softwood kraft (NBSK) pulp for fine paper manufacturing using cedar, hemlock and fir and used a CDEopCD bleaching process. The bleach sequence symbols are explained in Section 2.2.2. The effluent treatment was an activated sludge UNOX process. The UNOX process uses oxygen instead of air for aeration to achieve a high rate oxygen activated sludge system. This effluent was used throughout the batch study for all mini-studies, and will be referred to as Western Pulp effluent.

The second effluent was collected on January 5, 2006, from the Howe Sound Pulp and Paper Ltd. Mill in Port Mellon, British Columbia and was also treated whole mill effluent. This mill also produces NBSK pulp, using hemlock and cedar, as well as newsprint, which is pulped via thermo-mechanical process using a variety of spruce, pine, fir, hemlock and balsam. The bleaching process used is ODEopDnD; where n stands for nitrogen compounds. The remaining symbols are detailed in Section 2.2.2. This mill also uses the activated sludge UNOX process for effluent treatment. This effluent was only used for the adsorption rate kinetics, biological inhibition and isotherm mini-studies and will be referred to as the Howe Sound effluent. In addition, the practical application study took place at this mill.

Both of these effluents were grab samples collected on one occasion and stored in the Environmental Engineering Laboratory cold room at 4°C at the University of British Columbia for the duration of the study. No further preservation was used.

4.1.2 Methods and materials

Numerous test methods where used within each phase. Table 9 details these test methods and the materials used. All glassware and containers were washed with detergent, then rinsed with tap water and, finally, rinsed three times with distilled water. All glassware, such as Petri dishes and flasks used for growth of biomass, was also washed and rinsed similarly to the other glassware but with the addition of autoclaving for 30 minutes (min) at 122 °C and 124 kilo Pascal (kPa). Prior to use, all effluent samples were filtered with a 1.5 micrometer (μm) fiberglass filter paper, except the effluent used in the Molecular Weight Distribution and Practical Analysis Studies.

4.1.3 QA/QC

In order to ensure quality in measurement, every test performed was duplicated to avoid gross error. In addition, unless warranted by consistent results, every batch test was also duplicated. Blanks and/or reagent blanks were also analyzed for each parameter. In addition, all measurements of quantities removed were compared against experimental blanks that had been processed the same way as the samples.

Table 9. Methods and materials

Parameter	Method	Materials	Standards	Notes
Colour	HACH Method 8025 – Platinum Cobalt Standard Method with pH adjustment following Standard Method 2120 C – Spectrophotometric. Samples are centrifuged and filtered with 0.22 μm filter.	Filter apparatus and paper, H_2SO_4 at conc. so that amount does not exceed 3% of sample, DR2000 spectrophotometer	Platinum-cobalt Fisher Chemical 500 standard. Standards at 50, 100, 300 and 500 for 465 nm.	Measured at pH of 7.6 and at 465 nm in accordance with Pulp and Paper Technical Association of Canada standards.
pH	Standard Method 4500-H^+ B. – Electrometric Method	pH meter, buffer solution materials	pH 4, 7 and 10, Fisher Chemical	
COD	Standard Method 5220 D – Closed Reflux Method, Hach digester Method 8000 (EPA Approved) and DR/2000	Hach COD digester, 0.25 N of $K_2Cr_2O_7$, Ag_2SO_4 reagent,	800, 500, 300, 100, 50 and 0 COD as mg/L.	Make calibration curve @ 600 nm.
Electrical conductivity	Standard Method 2520 B. – Electrical Conductivity Method	Electrode, 0.01 M of KCl for standards	Zero and one standard @ 1412 μmhos/cm	
Molecular size distribution	Different membranes and TOC analyzer. Amicon stir cell 8200. Micropore YM 500, 1000, 3000, 10000, and 100000 Dalton membranes.	Amicon Stirred Cell and different membrane sizes.		Filter effluent through each membrane sequentially beginning with the largest and filtering the effluent passing with the next largest membrane. Measure organic content of each fraction with TOC measurement.
Total/Dissolved Organic Carbon	Standard Method 5310B – High Temperature Combustion Method	Shimadzu TOC analyzer	50 and 500 mg/L of C.	Acidify sample to pH < 3 first for DOC
Biochemical Oxygen Demand	Standard Method 5210B – 5-Day BOD Test	60 mL incubation bottles, nutrients, dilution water, dissolved oxygen meter, nitrification inhibitor	Dilution water blanks	
Total Suspended Solids	Standard Method 2540D – Total Suspended Solids Dried at 103-105°C	Drying oven, scale, filter apparatus, Millipore type AP40 filter	Distilled water filter blank	
Chloride	Standard Method 4500G – Mercuric Thiocyanate Flow Injection Analysis	Lachat flow injector	0, 50, 75, 100, 200, 400 mg/L	

4.2 Effluent Characterization and Biomass Production

4.2.1 Effluent characterization parameters

Table 10 lists the parameters that were analyzed for characterization of effluent.

Table 10. Parameters analyzed and rationale

Parameter	Rationale
Colour	Determination of process efficiency.
pH	Effluent pH can alter biosorption efficiency.
COD	Indicates organic content.
Electrical conductivity	Potential factor in biosorption efficiency.
Organic carbon	Quantifies molecular size fractions.
Molecular size distribution	Identification of colour imparting substances.

The methods and materials used were presented in Table 9.

4.2.2 Molecular weight distribution

In order to better understand the composition of the effluent, as well as to determine which fraction of the colour and COD is removed through dead *A. niger* biosorption, molecular size distribution was determined. Molecular weight distribution was performed as described in Table 9. Lignin and its derivates are generally high molecular weight compounds, therefore, the molecular weight distributions of the effluent should show this tendency (Sjostrom, 1981). In addition, certain removal mechanisms have tendencies to remove specific molecular weight fractions. For example, in a study done for the US EPA by Dugal et al. (1974), effluent characterization before and after lime treatment was performed and it was found that the lime process removed the higher molecular weight molecules. Therefore, the molecular size distribution can give insight into the potential removal characteristics of the dead *A. niger* biomass.

4.2.3 Biomass production

Fungus *Aspergillus niger* was produced and harvested in preparation for the batch and practical application study. A freeze-dried culture of *A. niger* was ordered from the American Type Culture Collection (ATCC), ATCC #11414. Biomass production included the following steps:

1. cultivation of biomass on agar plates,
2. cultivation of biomass in a liquid medium, and

3. harvesting biomass.

All culturing and harvesting steps were performed using aseptic techniques. All media and glassware was autoclaved for 30 min at 122°C and 124 kPa before use.

The freeze-dried culture required activation, which was performed by immersing the culture in distilled-deionized autoclaved water for 45 min. The solution was then transferred to potato dextrose agar plates, pouring approximately 10 milliliters (mL) of solution per plate. The plates were incubated for 7 to 10 days at 22°C upside down. Cultures from the incubated plates were transferred by streaking to fresh plates. This occurred once before the culture was used for biomass cultivation in the liquid medium. The cultures were maintained on agar plates for the duration of both Batch and Practical Application Studies. The plate cultures were refrigerated at 4°C where they were maintained for up to 2 months, at which time the culture was transferred to fresh plates.

The biomass to be harvested was cultivated in a liquid medium for four days. The liquid medium contained the following constituents: dextrose – 20 g/L (grams/litre); peptone – 10 g/L; yeast extract – 3 g/L. Two-litre Erlenmeyer flasks, containing 1 litre (L) of liquid medium, were inoculated with the fungus from the agar plates. The flasks were covered with a plug to protect from contamination while allowing aerobic conditions and placed on a rotary shaker at 125 rotations per minute (rpm) at room temperature (approximately 20°C). Each biomass culture was matured for four days.

After the culture had aged for four days the biomass harvesting commenced. Initial separation of the biomass from the growth medium was achieved by filtering the biomass and liquid medium mixture through a 150 μm sieve. The initial biomass washing regime consisted of a distilled water wash, to remove any remaining medium, followed by autoclaving the cells for 45 minutes. The distilled water wash consisted of washing the biomass over a 150 μm sieve with distilled water until the pH of the rinse water equaled that of distilled water. However, the observance of excessive COD initiated a mini-study of into the washing methods. This mini-study and results will be described in more detail in Section 4.3.3. As a result of the mini-study there was a switch to a double wash protocol that consisted of the original wash regime being followed by a second wash after the cells were autoclaved for 45 minutes.

Following washing, the biomass was dried for 36 h at approximately 60°C. After the biomass was dried, it was ground to a powder with an electronic coffee grinder. Particles that passed through a 250 μm sieve were used.

In order to ensure the pre-treated biomass was no longer viable, a portion of the finished pre-treated biomass was returned to an agar plate and incubated, as explained in the preceding section.

4.3 Batch Biosorption Study

The batch biosorption study consisted of eight key mini-studies on the following:

1. optimum pretreatment method,
2. effluent pH,
3. biomass washing method,
4. mixing requirements,
5. adsorption rate kinetics,
6. biological inhibition,
7. equilibrium isotherms,
8. removed fraction, and
9. temperature effect.

For each batch study the biomass was separated from the effluent by centrifuging the sample at approximately 3,500 rpm for 15 min, followed by filtering the sample with a 0.22 μm membrane filter. Originally, a 0.45 μm membrane filter was used, however, the sample turbidity interfered with colour measurement and therefore finer filtration was required. The Howe Sound effluent almost always required more than one filter paper per sample. For example, for the same dose and same biosorption time, the Western Pulp 45 mL centrifuged sample only required one filter paper for this volume, whereas the Howe Sound effluent normally required at least two. As a result, there was potential for more variability in the Howe Sound results since more processing was required. As a note, in order to be properly compared, the experimental blanks were also filtered using two papers for Howe Sound effluent experiments.

4.3.1 Pre-treatment mini-study

The purpose of the pre-treatment mini-study was to determine which pre-treatment yielded the highest colour removal efficiency. Subsequent to harvesting biomass, the pre-treatments were applied. In total, eight different methods of pre-treatment were utilized. The eight biomass pre-treatment methods included the following:

1. Type A - autoclaving for 30 minutes at 122°C and 124kPa and then biomass was dried at 60 to 70°C for 36 h in a drying oven,
2. Type B – contacting 100 g wet weight pellicles with 1L of 0.1 Molar (M) NaOH solution for 1 h at room temperature,
3. Type C – contacting 100 g wet weight pellicles with 1L of 0.1 M HCl solution for 1 h at room temperature,
4. Type D – contacting 100 g wet weight pellicles with 1L of 0.1 M H_2SO_4 solution for 1 h at room temperature,
5. Type E – contacting 100 g wet weight pellicles with 1L of 0.1 M $CaCl_2$ solution for 1 h at room temperature,
6. Type F – contacting 100 g wet weight pellicles with 1L of 0.1 M $NaHCO_3$ solution for 1 h at room temperature,
7. Type G – contacting 100 g wet weight pellicles with 1L of 0.1 M Na_2CO_3 solution for 1 h at room temperature, and
8. Type H – contacting 100 g wet weight pellicles with 1L of 0.1 M NaCl solution for 1 h at room temperature.

Type B thru H pre-treatments were also autoclaved and dried as listed in Type A.

After pre-treatment and prior to drying, the biomass was washed with distilled water until the wash water reached the original pH of the distilled-deionized water. The biomass was then dried and ground.

The pre-treatment batch mini-study was performed on the Western Pulp effluent and consisted of eight separate batch tests, each using 75 mL of effluent at original pH and 0.2 g of pre-treated biomass. Each pre-treatment method was tested twice, although in some instances the second trial was scaled down due to the amount of biomass that was recovered after drying. Experiment blanks were analyzed concurrently to determine the effects of this process on colour and COD.

The effluent and biomass was put into a 125 mL Erlenmeyer flask and placed on the shaker/incubator for 48 hours (h) at 125 rpm. After 48 h of contact, the mixture was centrifuged and then filtered with a 0.22 μm membrane filter under vacuum pressure.

The adsorption performance of Type A thru H pre-treated biomass was determined by analysis of residual colour in the filtrate at both 400 and 465 nm and COD. Colour was measured at two wavelengths since the highest measurement of absorbance in the visible spectrum on the raw effluent was at 400 nm. Therefore the results obtained at 400 nm showed colour that was not measured at 465 nm. Based on the results for colour removal, the optimum pre-treatment method, autoclave only, was selected and used in the remainder of the study.

4.3.2 Effluent pH mini-study

The pH mini-study illustrated the effect of pH on biosorption and gave an indication of optimal effluent pH ranges.

This mini-study was performed on Western Pulp effluent. Prior to adding the biomass to the effluent, the pH of the effluent was altered. The adjustment of pH was achieved using sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) depending on the pH target. The initial effluent pH was adjusted to the following pH levels 2, 4, 6, 8, 10, and 12. Batch tests were performed, each using 75 mL of one of the six pH-adjusted effluents and 0.2 g of the biomass in a 125 mL flask. Replicate batch tests were performed on each pH-adjusted effluent, resulting in 12 tests plus an experimental blank at original pH. These flasks were then placed on the shaker/incubator for 48 h at 125 rpm. After the 48 h of contact, the mixture was filtered with a 0.22 μm membrane filter under vacuum. Further, based on the results of this test, experimental blanks at pH 2, 4, 6, and 8, were performed in order to determine the quantity of removal that occurred as a result of the pH adjustment and how much removal was due to the biomass biosorption.

The adsorption performance at each of the initial effluent pH levels was determined by analysis of colour and COD. Although pH did have an effect on biosorption, it was determined that a more practical approach would be to perform the remaining studies using effluent at the original pH.

4.3.3 Biomass washing mini-study

An important part of the biomass harvesting process was the biomass washing step. The original biomass washing protocol was a single wash of the cells prior to autoclaving. However, through results obtained using the biomass, it became evident that the addition of the fungus to liquid resulted in an increase in dissolved matter in the liquid. Therefore, attention was turned to the fungal wash methods.

In order to determine the levels of colour and COD introduced to a liquid by the fungus, the prepared fungal biomass was added to de-ionized water in the same manner as during normal testing (ie, 75 mL of water, 0.2 g of biomass, on shaker at 125 rpm). These batch tests were sampled after different time intervals within a 2-day period, at 15 min, 24 and 48 h. An experimental blank of distilled-ionized water was used. Measurement of the colour and COD was used to determine the appropriate biomass washing method.

As the original biomass wash method was not sufficient, two other biomass wash methods were tested. One method involved autoclaving the biomass while still in the growth medium, followed by washing and draining of the biomass. This wash method is referred to as the "wash after autoclaving" method. The other biomass wash method consisted of washing the biomass both before and after autoclaving. This method is referred to as the "double wash" method. For both of these methods, washing was continued until the wash water pH was that of the distilled water.

Based on the values of colour and COD, it was found that the "double wash" method was the most effective and, therefore, it was used for preparation of the biomass for the reminder of this study.

4.3.4 Mixing mini-study

Due to observations of incomplete mixing in the original set of batch adsorption tests, it was determined that analysis of mixing efficiency was required. In the original mixing method, a flask size of 125 mL containing 75 mL of effluent at a rotational speed of 125 rpm was used.

It was determined that two main factors may affect mixing on a rotary shaker:

1. the relationship of flask size to amount of effluent it contains; and
2. the rotational speed of the shaker.

In addition, it was anticipated that the dose of fungus added to the effluent might also impact the mixing efficiency. Table 11 lists these variables and the ranges in which they may impact mixing. Note that the effluent amount in each test was 75 mL.

Table 11. Possible factors impacting mixing efficiency

	Variable 1 Shaker Speed (rpm)	Variable 2 Flask Size (mL)	Variable 3 Biomass Dose (g)
Setting 1 – Low	125	125	0.2
Setting 2 – Medium	160	-----	0.5
Setting 3 – High	200	300	0.8

Table 12 lists the experimental runs performed based on the variables listed in Table 11.

Table 12. Possible experimental runs

Run Number	Shaker Speed (rpm)	Flask Size (mL)	Biomass Dose (g)
1	125	125	0.2
2	125	125	0.5
3	125	125	0.8
4	160	125	0.2
5	160	125	0.5
6	160	125	0.8
7	200	125	0.2
8	200	125	0.5
9	200	125	0.8
10	125	300	0.2
11	125	300	0.5
12	125	300	0.8
13	160	300	0.2
14	160	300	0.5
15	160	300	0.8
16	200	300	0.2
17	200	300	0.5
18	200	300	0.8

For each experimental run, batch adsoprtion tests were carried out with adsorption times of 0.25, 1, 4 and 24 h. Effluents that had been shaking without the addition of fungus for 24 h were used as experimental blanks. Again, Western Pulp effluent was used for this mini-study.

There were 18 possible experimental runs using these combinations of factors. From prior testing, Run 1 was analyzed at times 0.25, 1, 4, and 24 h, as well as additional times, and, therefore, repeating this experiment was not required. Also, Runs 2 and 3 had been partially executed but with samples taken only at 48 h. Although analysis was not complete due to missed sampling times, these previous test results give some indication of the expected results. Based on the data previously obtained the following experimental plan was proposed.

1. Runs 10, 11, and 12 were undertaken to confirm if flask size was a factor. All of these tests were run simultaneously for 48 h only.
2. Runs 16, 17, and 18 were completed in order to determine if there was an impact on mixing efficiency between rotational speeds of 125 rpm and 200 rpm. All of these tests were run simultaneously.
3. Further testing would be completed if necessary, such as Runs 13, 14, and 15.

Based on the results obtained, runs 10, 11, 12, 16, 17, and 18 were performed. It was determined that using a flask size of 300 mL at 125 rpm was sufficient for complete mixing for all doses tested and, therefore, this flask size and rotational speed was used in all further testing. Each of the adsorption tests was performed twice and colour, COD, DOC and pH were measured.

4.3.5 Kinetic mini-study

The purpose of the kinetic mini-study was two-fold, to determine the rate at which the biosorption occurred, and to determine the equilibrium time for adsorption for the following isotherm mini-study.

This mini-study used 75 mL of effluent and 0.2 g of pre-treated biomass per flask. Experimental blanks using only effluent with shaking for 48 h were used, to determine the effect of this process on colour. Samples were collected at times 0, 0.08, 0.17, 0.25, 0.5, 0.75, 1, 4, 8, 12, 19, 24, 32, 40, 44, 48 and 52 h. This mini-study was performed on both Western Pulp and Howe Sound effluents, however, only the Western Pulp experiments were duplicated. The adsorption performance at each time was determined by analysis of colour, COD, DOC and pH.

The results of this mini-study were used to determine the equilibrium time beyond which no additional significant colour removal occurred. Further, the kinetics of the adsorption were studied by comparing the data with the Lagergren (1898) and Ho et al. (1996) equations.

4.3.6. Biological inhibition mini-study

Initial results from kinetic testing showed an additional peak of colour removal occurring at roughly 30 plus h, after the initial adsorption period slowed. The mechanisms that caused this delayed colour removal were not clear. It is possible that these mechanisms were chemical, biological or bio-chemical. In order to help identify what mechanisms were responsible, biological inhibitors were utilized to determine if the delayed colour removal effects were related to biological activity. As there was only one factor in this study, inhibition, only three tests sets were required. They are listed in Table 13.

Table 13. Biological inhibited test sets

Test Set #	Inhibitor
1	Environmental temp < 4°C
2	Addition of Sodium Azide
3	Addition of Sodium Fluoride

The test sets were sampled at set intervals of 0, 1, 8, 19, 24, 32, 40, 48, and 52 hour for test set 1 and the same intervals, less the 19 and 52 hour intervals, were used for test sets 2 and 3. Colour, COD, DOC and pH were analyzed for each sample. In test set 1, the incubator and the effluent was at 4°C before commencing the test set. For test sets 2 and 3, chemical addition of sodium azide (NaN_3) and sodium fluoride (NaF) at 1 milli-molar (mM) and 50 micro-molar (μM), respectively, was used based on information found regarding doses of these chemicals for biological inhibition (Carlson and Suttie, 1967; Desseaux et al., 2002; Hongslo et al., 1974; Rachenko et al., 2004; William Ramey, University of British Columbia, personal communication, January 15, 2006; Vyas and Molitoris 1995). These chemicals were added to the solution and mixed for 15 minutes before the addition of the biomass.

All three test sets were performed on Western Pulp effluent. Based on the results of these two methods, set 1 and 2 were applied to the Howe Sound effluent in order to obtain kinetic data results for both effluents that were not influenced by biological activity. Test set 1 for both effluents was duplicated, however, test set 2 was not duplicated as it was felt that the results were

consistent with the other test and duplication was not required.

4.3.7 Isotherm mini-study

The isotherm mini-study provided a description of the adsorption characteristics and information about biomass doses required. The experimental time for this mini-study was determined by the results of the kinetic study. Therefore, each isotherm adsorption period was 32 h.

Unlike previous mini-studies, this mini-study used biomass amounts of 0.1, 0.5, 1.0, 2.0 and 3.0 grams to 75 mL of effluent, which gave doses of 1.33, 6.67, 13.33, 26.67 and 40.00 g/L. These tests were run with effluent inhibited by NaN_3 at 1 mM. Both Western Pulp and Howe Sound effluents were tested once. In addition, an experiment blank using effluent was run for each set of batch tests. The samples were tested for colour, COD, DOC and pH. The data were compared to three isotherms, the Langmuir, Freundlich and BET.

4.3.8 Removed fraction mini-study

In order to better understand the biomass removal, molecular weight distributions were determined again, however, this time on the effluent samples of the isotherm mini-study. Both the effluent blank and 1.0 g biomass batch test samples were fractionated using the Amicon stir cell and membrane. In this mini-study only 3,000, 10,000 and 100,000 Dalton (Da) membranes were used. This mini-study detailed the fractions being removed or added by the addition of 1.0 g of biomass to 75 mL of effluent.

4.3.9 Temperature mini-study

In the inhibition mini-study, a biologically hindering ambient temperature was used as an inhibitor. It was observed that batch tests run at 4°C showed less removal than tests at room temperature, i.e. 20°C. In order to better understand this occurrence, batch studies were performed at 35°C, similar to the kinetic rate mini-study, at time intervals 1, 8, 20, 24, 32, 40, 48 h. The effluent was inhibited with NaN_3 at a 1 mM concentration. These results were compared with the results of the kinetic study.

4.4 Practical Application Study

After completing the Batch Study and determining that the fungus was successful under controlled and optimum conditions, a simulated real world application was tested. The practical application testing was conducted on-site at the Howe Sound Pulp and Paper Partnership Ltd. pulp mill on February 22, 2006.

In order to mimic the activated sludge system used at the plant, samples were collected from the primary clarified effluent line as well as from the return activated sludge (RAS) line. Due to extremely high dosing of nutrients to the activated sludge system in the days prior sampling, addition of further nutrients beyond what already existed in the RAS sample was not required.

The experiment, although intended to be a scaled down replication of the existing continuous activated sludge system at the mill, was tested using a batch process. Five batch tests ran simultaneously in 500 mL Erlenmeyer flasks. Each flask was sealed with a bung that had an air input and output. Air was supplied by aquarium bubblers and dispersed by small diffusers that hung, by tubing, down from the bung submerged in the RAS/effluent mixture. Each batch test contained 300 mL of a mixture of effluent and RAS at the same ratio as used by the mill, 100:55, i.e. 194 mL of effluent with 106 mL of RAS (Siew Sim, personal communication, February 22, 2006). Each flask was also mixed with a magnetic stir bar. This was to ensure complete mixing occurred, which may not have been achieved by aeration alone.

The average residence time of each step in the mill's activated sludge process was used for the batch test. The mill's activated sludge system had a four to six hour residence time in the aeration chamber followed by eleven to twelve hours of settling (Howe Sound Pulp and Paper Ltd. Partnership, 2006; Siew Sim, personal communication, February 22, 2006). After the biomass was added, aeration was started immediately and continued for 4 h. After the aeration was terminated, the activated sludge was left to settle for 12 h. After settling, the samples were decanted and put on ice until transported to the University of British Columbia, Environmental Engineering Laboratory for analysis.

Of the five batch tests, two were experimental blanks or controls that contained only the effluent and RAS. The other three were dosed with fungal biomass in one of three quantities, 0.8, 4.0 and 8.0 g, which were equivalent to dosages of 2.67, 13.33 and 26.67 g/L, respectively.

To determine the effect of the biomass on the treatment system, COD, BOD, TOC, TSS and colour was measured. The removal efficiencies of the controls were compared to the results obtained when adding the fungus. Although this testing was very crude, it would indicate potential for use in the activated sludge process.

5. RESULTS AND DISCUSSION

5.1 Effluent Characterization and Biomass Production

5.1.1 Effluent characterization

In order to gain a better understanding of the characteristics of the effluents, both were analyzed for colour, COD, DOC, pH, electrical conductivity, and chlorides. The results of this analysis are listed in Table 14.

Table 14. Results of whole mill treated effluent characterization

	Western Pulp	Howe Sound
Colour (TCU)	1750	1050
COD (mg/L)	370	310
DOC (mg/L)	190	140
BOD (mg/L)*	12.1	10.8
pH	7.6	7.4
EC (μS)	2100	3000
Chlorides (mg/L)	156	146

* Yearly average data obtained from the plants

Colour was obviously a very important factor as it was the focus constituent of the study. Comparing the colour of the Western Pulp and the Howe Sound effluent shows that the Western Pulp whole mill effluent was significantly higher in colour. This could have been a result of the pulping and bleaching processes used, process efficiency and/or wood type. As northern softwood was used at both mills, using generally the same species of wood, it was unlikely that wood type would have a significant impact on colour. More likely, this difference was a result of the pulping and bleaching processes and their efficiencies.

Howe Sound employs two pulping processes, kraft and thermo-mechanical, as opposed to Western Pulp, which used kraft pulping only. Thermo-mechanical pulp is used to make newsprint and, therefore, was not bleached. The kraft process, which produces a physically stronger pulp, does not remove colour very effectively and, therefore, a bleaching step is required (Fengel and Wegner, 1984). As the bleaching process is the major contributor of coloured effluents, it was likely the thermo-mechanical effluent had a small impact on the amount of colour in the effluent stream through dilution of colour from the kraft stream.

In addition, the bleaching sequence can also affect the colour of the effluents. Based on real plant data, bleach sequences using oxidative species instead of chlorine produce significantly less effluent colour (Springer, 1986). The Howe Sound mill uses an ODEopDnD non-elemental chlorine sequence as opposed to the Western Pulp mill that used a CDEopCD chlorine based sequence. As noted previously, the major contributor of coloured effluents in a pulp mill is the bleaching system. As such, it was conceivable that the difference in bleaching sequence had a significant impact on the difference in colour.

Lastly, the Howe Sound mill's pulping and bleaching processes are more modern than Western Pulp's and, therefore, runs "tighter" mill operations. This could have impacted the reduction of colour in the mill's effluents.

The other constituents measured, similarly to colour, also showed the Western Pulp effluents having higher levels than Howe Sound, with the exception of electrical conductivity. This supports the theory that the bleaching processes and the mill's ability to run a "tight" operation are differentiating factors in the condition of the effluent.

The COD and DOC values can indicate the organic loading of the effluent and what fraction is recalcitrant. From data supplied by the mills, the yearly BOD averages for Western Pulp in 2004 and Howe Sound in 2005 were 12.1 mg/L and 10.8 mg/L, respectively (Howe Sound Pulp and Paper Ltd. Partnership, 2006; Jeanne Taylor, electronic mail, May 4, 2005). Therefore, it was assumed that most of the organic matter in both of the effluents was recalcitrant. Electrical conductivity, pH and chlorides are all parameters that have the potential to affect biosorption. Therefore these factors may impact the biosorption process occurring in a specific effluent.

5.1.2 Molecular weight distribution

Using the Amicon stir cell and various sized membranes, a molecular weight size distribution was determined. It should be noted that, unlike the effluent characterization, the samples were not pre-filtered with a 1.5 μm fiberglass filter paper prior to the analysis. Figures 1 and 2 summarize the colour and TOC of each fraction for the Western Pulp and Howe Sound effluents.

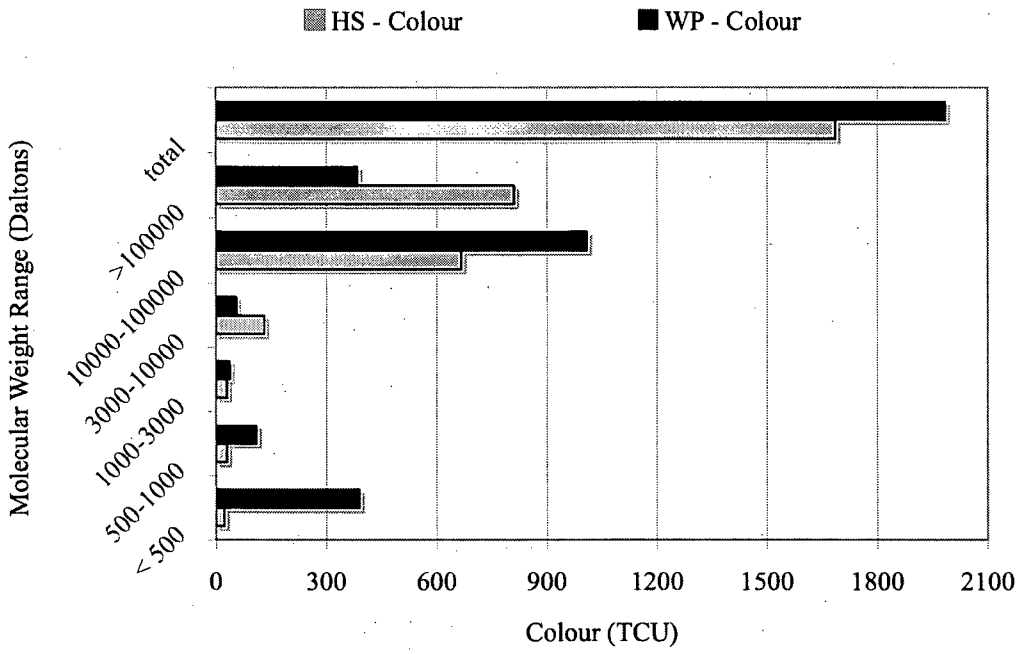

Figure 1. Molecular weight distribution by colour

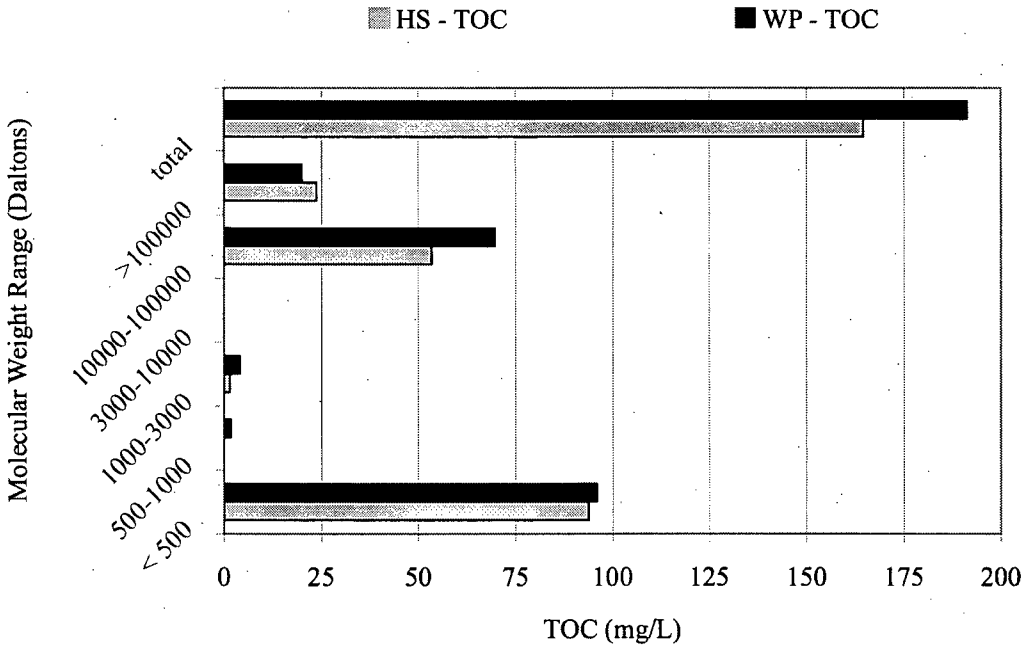

Figure 2. Molecular weight distribution by total organic carbon

The results of the molecular weight study shed light on the distribution of chromophores in the effluents. As opposed to the Howe Sound effluent, which contained predominantly molecules that were larger than 10,000 Da, the Western Pulp effluent contained two predominant ranges of high and low molecular weight colour components. These results were consistent with the generally accepted theory that the colour was primarily composed of lignin-related compounds, since they are typically higher molecular weight compounds (Sjostrom, 1981). In addition, both high and low molecular weight recalcitrant organic compounds have been found in pulp mills (Konduru, et al., 2001).

The difference in colour distributions was possibly related to the variation in bleaching sequence and the effluent minimization strategies used by the plants. However, the colour distribution data also indicated that dilution by the thermo-mechanical pulping effluent stream is not a significant factor. Both of these assumptions were based upon the substantial difference in colour distributions. If dilution by the thermo-mechanical stream was a major factor, the Howe Sound colour distribution would be more similar to the Western Pulp, albeit at smaller concentrations. On the other hand, the molecular weight distributions of the organic matter, TOC, were quite similar for the two effluents. This did not allow for clear conclusions regarding the source of the effluent colour difference and made the previous assumptions based on the colour distribution questionable.

This raised issues regarding colour measurement itself. Colour measurement of a liquid is a function of light absorbance, not a specific measurement of the amount of chromophoric materials. Therefore, unlike measurement of a specific compound such as carbon where it is a direct function of the quantity of the material, the quantity of coloured materials is not measured but is instead the amount of light those materials absorb. So, in comparing two different single molecules, one may be a "stronger" chromophore than the other and, therefore, have a higher absorbance which results in a higher colour reading. Nevertheless, the quantity of the material could be the same for each coloured molecule. This means that it is difficult to make conclusions regarding sources of colour since the strength of the chromophores is also a factor. The Western Pulp mill's pulping and bleaching sequence may have created stronger chromophores in the low molecular weight region that were not formed by the Howe Sound pulping and bleaching processes or those absent chromophores were recycled.

5.1.3 Biomass production

The *A. niger* biomass grew easily both on the plates and in the liquid medium. Three streak plates were maintained at any one time to ensure the continuous supply of biomass for inoculation of the liquid medium for biomass production. On the plates, the biomass exhibited a whitish-creamy colour mass or mycelium, with black spores-head on topside only. These characteristics were consistent with descriptions of *A. niger* given by Olds (1975). Illustration 2 is a photograph of a plate.

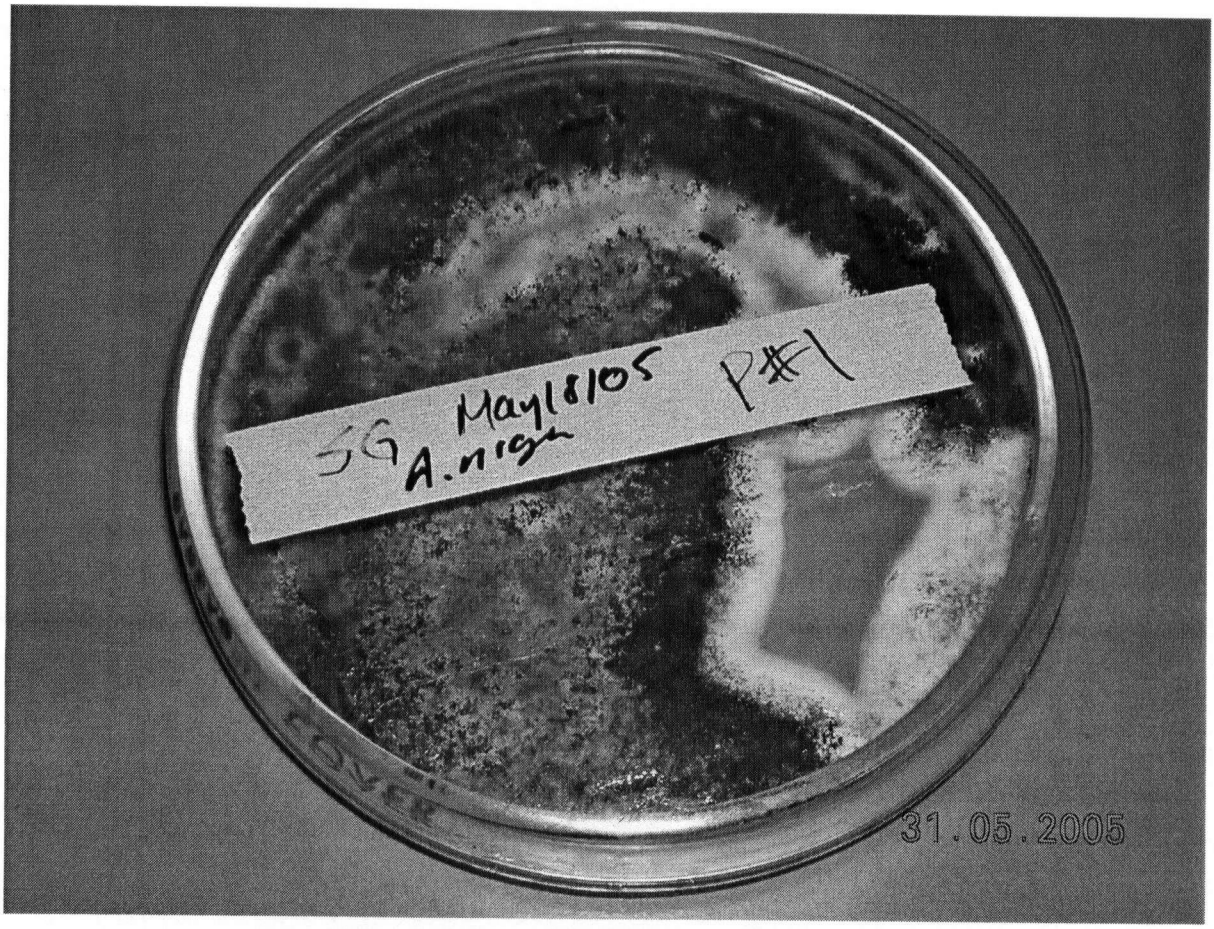

Illustration 2. Photograph of *A. niger* agar plate

In order to further clarify the identity of the fungal species, the biomass was observed under a microscope at an optical magnification of 100 times (100x). Illustration 3 is a microscope image of broken conidiphore and conidia of the biomass. The conidiphore was broken as a result of poor slide preparation techniques.

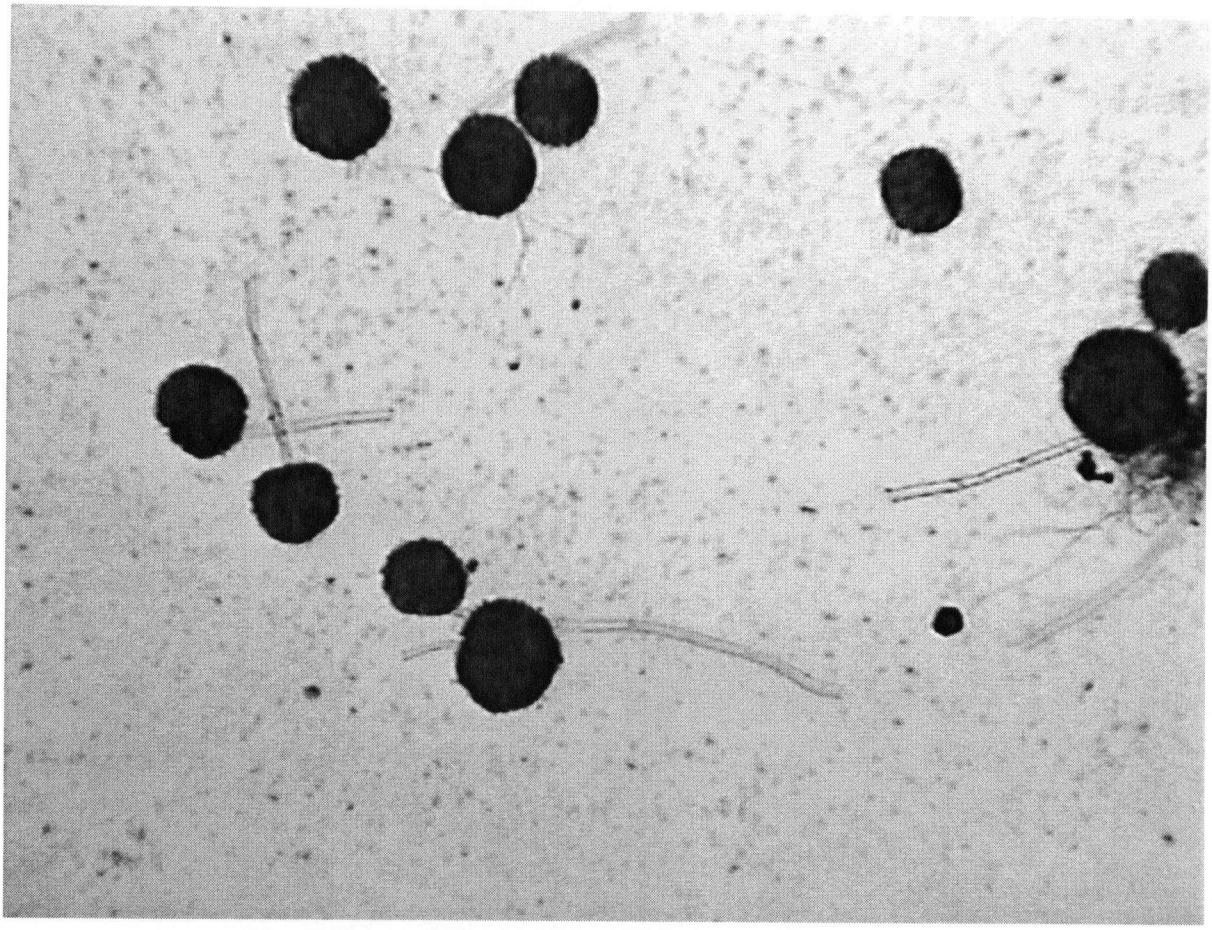

Illustration 3. Microscope view of conidia at the end of broken conidiphores (100x)

This microscope image verifies the biomass as *A. niger*.

The biomass used for biosorption testing was produced in liquid medium and formed white-creamy coloured globules approximately two centimeters in diameter. Illustration 4 is a photograph of the biomass prior to harvesting.

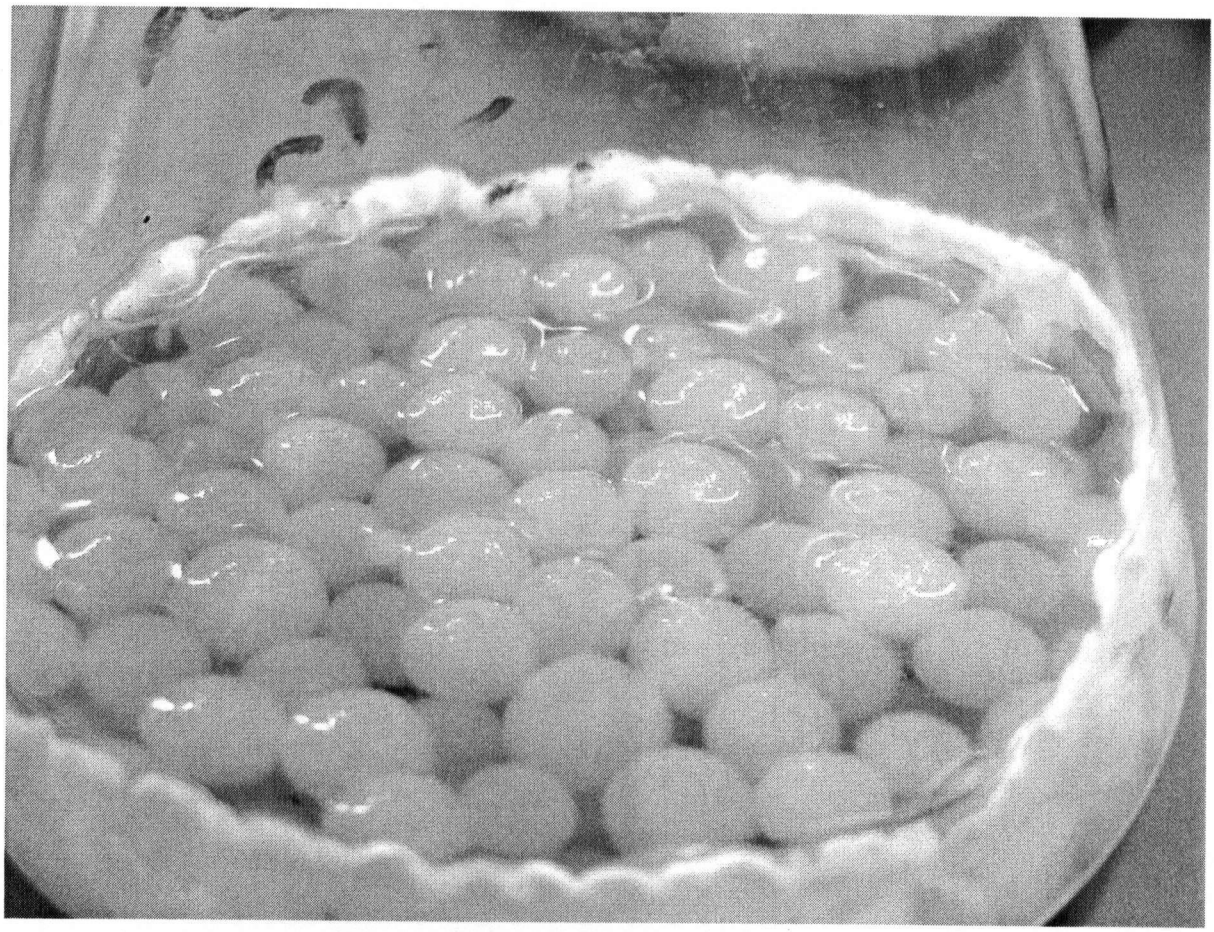

Illustration 4. Biomass in liquid medium just prior to harvesting

The biomass was grown in the liquid medium for four days and was then harvested. The biomass was then dried and inactivated by autoclaving for 45 min. In order to ensure that the biomass was in fact not viable after autoclaving for 45 min, biomass that was pretreated and dried was smeared onto agar plates of potato dextrose agar and incubated for 7 days. Of the two such smear plates that were prepared neither showed regrowth of biomass. Illustration 5 is a picture of one of the smear plates.

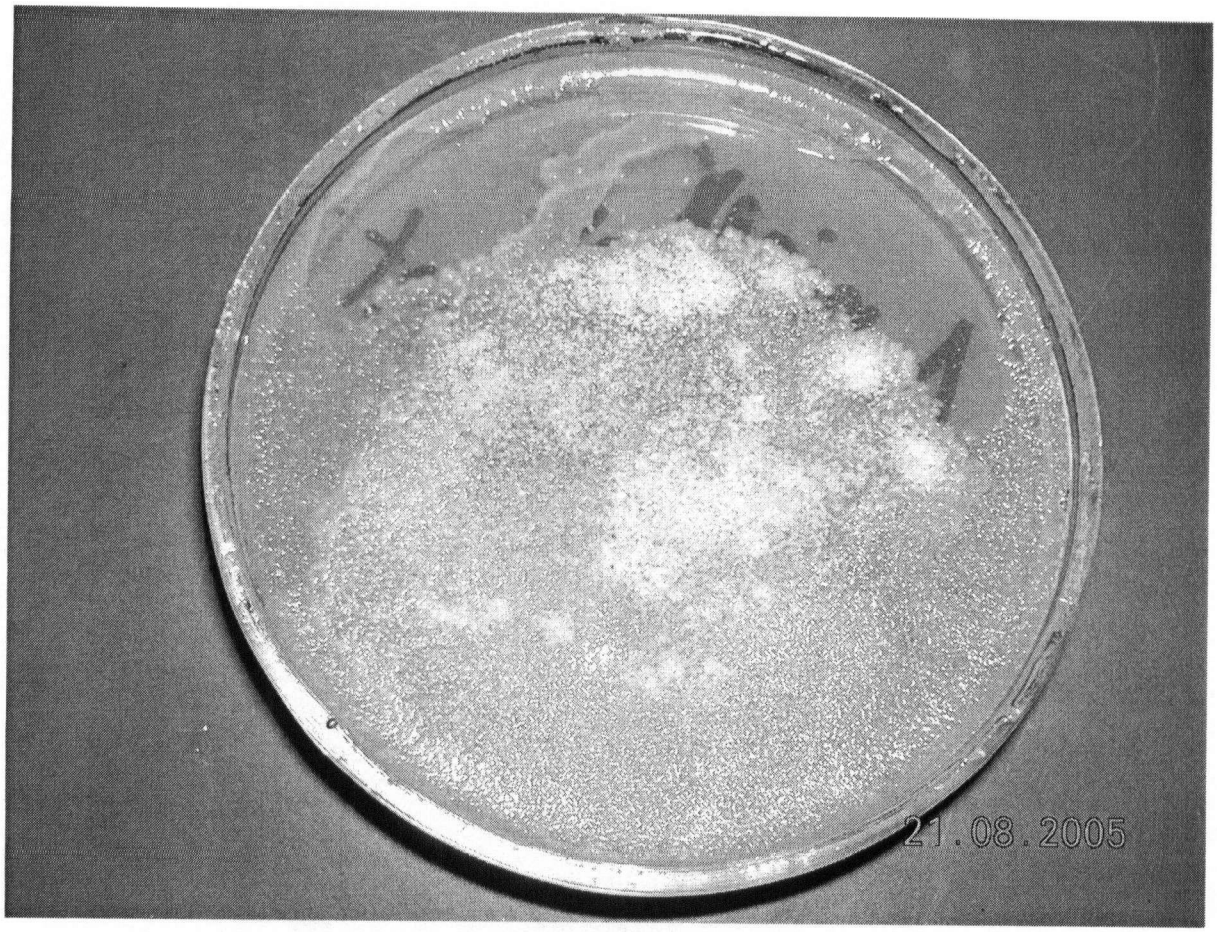

Illustration 5. Inactivated biomass smear plate.

5.2 Batch Biosorption Study

Due to the large amount of data obtained within the batch biosorption study, only data vital to the discussion will be presented within this Section. Other data obtained are provided in the Appendices.

5.2.1 Optimum pretreatment mini-study

The first mini-study was used to determine the optimal biomass pretreatment method. Colour was measured at both 400 and 465 nm, since at 400 nm, the total absorbance of the original effluent was higher than at 465 nm. Figure 3 and 4 displays the colour removal efficiencies at 465 nm and 400 nm, respectively, for the various pre-treatment methods on Western Pulp effluent.

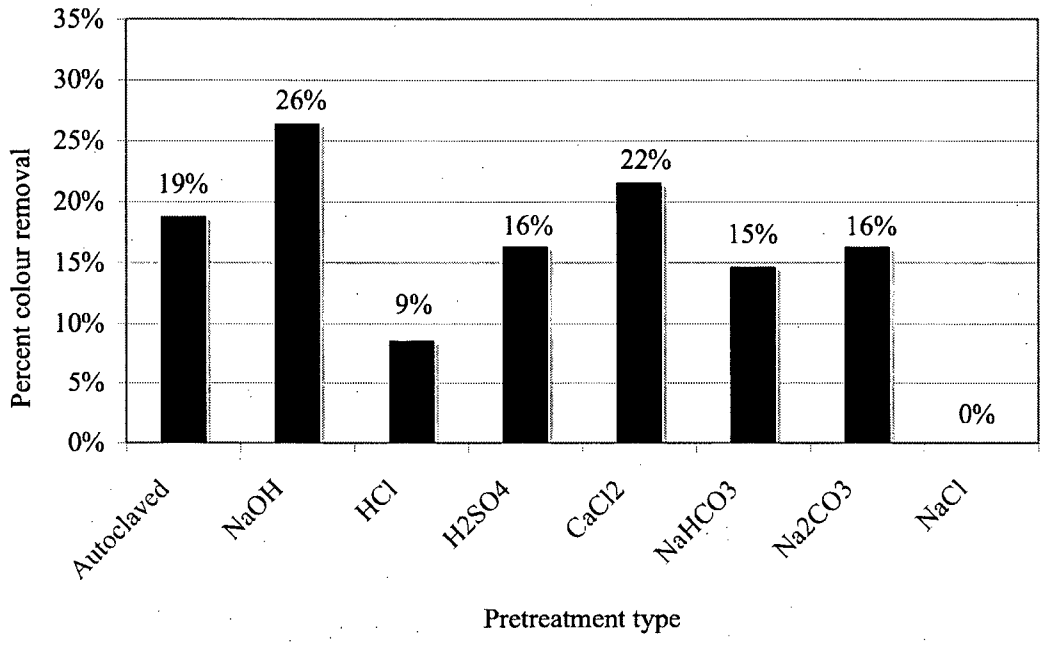

Figure 3. Colour removal efficiencies from WP effluent at 465 nm for various pretreatments after 48 h of contact

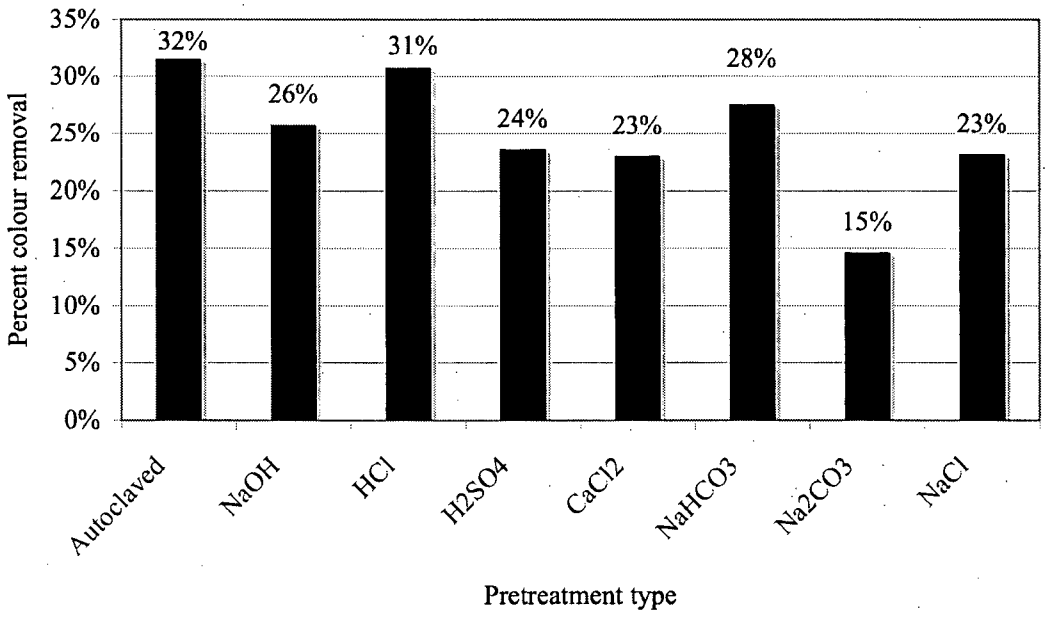

Figure 4. Colour removal efficiencies from WP effluent at 400 nm for various pretreatments after 48 h of contact

The above data directed attention to the difference in absorbance from the same samples measurement at different wavelengths. It is important to note that for each batch test, two colour samples were taken and these same duplicates were both measured at 400 and 465 nm. Again, issues of colour measurement were raised. Figures 3 and 4 illustrate the percentage colour removal efficiencies, but, they do not indicate the difference between the experiment blank readings at 400 and 465 nm, which produced absorbances of 0.258 and 0.152, respectively. These experiment blank results were used as reference points from which to measure percent removal in order to analyze the pretreatment efficiencies. Again, it must be noted that colour measurement is not a measure of quantity of chromophores but a measure of absorbed light. It was concluded that light at 400 nm was absorbed more readily by the chromophores in the pulp mill effluent than that at 465 nm.

When analyzing which pretreatment method was most appropriate for use in this research, the values obtained at 400 nm had more significance than those at 465 nm, since the 400 nm values represented the removal of more colour. Although the removal efficiencies after pretreatments with NaOH, HCl, $CaCl_2$, and $NaHCO_3$ were notable, ultimately the autoclaving-only pretreatment was selected for further use. This was primarily due to the simplicity of this pretreatment process and the choice was reinforced by the lack of obvious superiority of any of the other pretreatments. The autoclaving-only pretreatment did not involve the use of chemicals or additional soaking time, but only involved autoclaving of the cells, which was also required with every pretreatment process. This is more practical for real world application.

Other studies using inactive biosorbents have also selected autoclaving only for pretreatment. Zhou and Banks (1991), for use on humic acids, and Gallagher et al (1997), for use on dyes and metals, found that autoclaving the biomass increased biosorption. For removal of Basic Blue 9 dye, Fu and Viraraghavan (2000), selected autoclaving only pretreatment for further use in that study. Fu and Viraraghavan, also found that NaOH, $CaCl_2$, and Na_2CO_3 provided similar removal efficiencies to the autoclave-only pretreatment. In fact, generally there were many similarities in the effects of pretreatment on biosorption between Fu and Viraraghavan's study on Basic Blue 9 and the present study results at 400 nm, with a few distinct differences. NaOH pretreatment has also been used by Banks and Parkinson (1992), and Zhou and Banks (1993), for humic acid removal, Gallagher et al. (1997), for use on dyes and metals, and, Rao (2001), for

removal of phenol, and was found to provide increased removal efficiency. In another study by Fu and Viraraghavan (2002), on Congo Red dye, pretreatments with HCl, H_2SO_4, $CaCl_3$ and $NaHCO_3$ were the most effective, with $NaHCO_3$ pretreatment being selected for use. Like the Basic Blue 9 dye pretreatment results, there were many parallels between the pretreatment results of the Congo Red and the current study.

The data obtained in this mini-study provided a great deal of information regarding the chromophoric material and biosorption using *A. niger*. Based on the results of colour absorbance at 400 nm, it was possible to assume that the effects of electrostatic adsorption were not significant for removal of the chromophores, since the pretreatments generally seemed to slightly reduce biosorption rather than increase it when compared to autoclaving-only pretreatment. HCl and $NaHCO_3$ pretreatments showed similar removals to autoclave-only pretreatment for measurement at 400 nm; these pretreatments were used with the intent of altering the fungal surface charge of the fungal biomass which is typically negative (Fu and Viraraghavan, 2001b).

NaOH is used to expose chitin in the fungal cell walls and, since this pretreatment enhanced the removal of colour that absorbs at 400 and 465 nm, it can be assumed that the colour was biosorbed on chitin sites.

Colour removal efficiencies at 465 nm provided similar conclusions for most effective pre-treatment, although it was apparent that the chromophores absorbing light at this wavelength were removed by slightly different mechanisms, since some pretreatments were more successful at removing colour bodies reflected at 400 nm. Again, autoclaving and NaOH pretreatments showed substantial removal, which indicated a physical type adsorption not dependant on electrostatic surface charges.

Evident from the results of the molecular weight distribution measurements, the effluent colour was composed of a wide variety of molecules. Therefore, defining the mechanisms responsible for the removal of the chromophores was difficult since many may have been working at once. The different chromophores in the effluent could have been removed by different biosorption mechanisms.

5.2.2 Optimum pH mini-study

The alteration of the effluent pH did have an effect on the biosorption capability. Figure 5 displays the results of initial pH on colour removal from Western Pulp effluent. "Total" refers to removal of colour through the entire process of pH adjustment and biosorption, and "fungus only" describes the removal that occurred only due to biosorption, i.e. subtracting the removal associated with pH adjustment from the overall removal efficiency. In every situation pH was adjusted to experimental pH, the experiment was performed, the sample was filtered and finally the pH was adjusted to pH 7.6 prior to colour measurement.

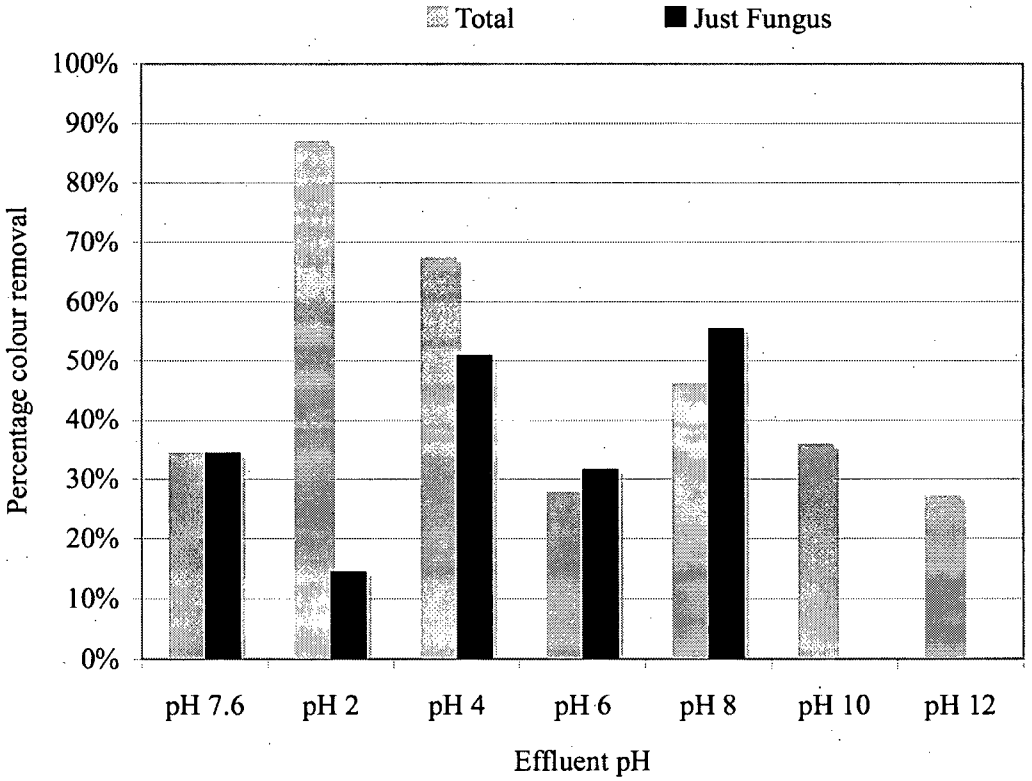

Figure 5. Effect of initial effluent pH on biosorption

The initial effluent pH not only altered the biosorption efficiency but also the colour quantity in the effluent after filtration. For example, by adjusting the effluent pH to 2, 70% of the colour was removed without biosorption. This indicated that a reduction of pH to very low values precipitated some of the chromophoric material, which then was filtered from the samples. This

also occurred at pH 4. The reverse happened at pH 8, whereby the colour bodies became more soluble and, therefore, colour that would have previously been filtered by the removal of the biomass was not removed. Essentially, the biomass was exposed to more colour and thus appeared to be more successful.

There is no clear trend regarding the effect of the initial effluent pH on the biosorption efficiency. Typically, biosorption occurs more readily under either basic or acidic conditions but generally not both. However other variations have been reported (Fu and Viraraghavan, 2001a; Rao, 2001; Zhou and Banks, 1993). Again, the reason that a clear trend was not seen for the effluent colour could be a result of the "blend" of chromophores in the effluent. It appeared that pH 4 and 8 were the most effective initial pHs. This could have been related to an electrostatic mechanism of biosorption. Neutralization of the negative surface charges could have enhanced the removal at pH 4.

5.2.3 Biomass washing mini-study

Due to the observance of excessive COD levels in the filter effluent in the previous batch tests, the biomass washing mini-study was performed. This study involved addition of the biomass to distilled-deionized water. The biomass and distilled-deionized water mixture was shaken on the rotary plate for the specified time periods. COD was measured from the biomass and water mixtures after filtering with the 0.22 μm filters.

Figure 6 displays the results of this study using Western Pulp effluent. Please note, as stated in Section 4.3.3, "wash before autoclaving" means wash before autoclaving only, "wash after autoclaving" means wash after autoclaving only and "double wash" means washing before and after autoclaving.

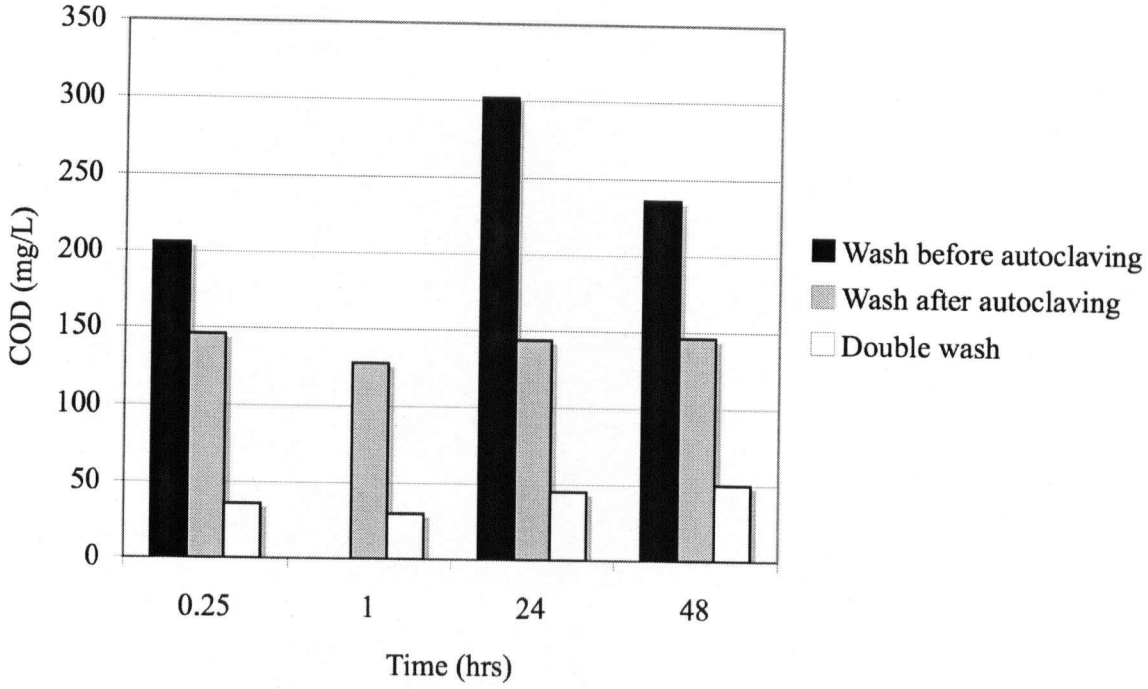

Figure 6. Biomass wash study results where biomass was in contact with distilled-deionized water

One of the duplicate test results was removed for time 1 h for the "wash after autoclaving" test, since it was more than 100% higher than the other sample taken at this time and the rest of the wash after results. A sample at time 1 hour was not taken for the "wash before autoclaving" test.

From Figure 6 it is quite evident that the "double wash" method was the most effective as the COD results showed the double wash method filtered samples to have significantly less COD after contact with biomass in all the specific time periods. Further the "wash after autoclaving" method also proved to be more effective than the originally utilized "wash before autoclaving" method since the COD concentrations remaining in the filtered samples were higher than with the "wash before autoclaving" method.

With the "wash before autoclaving" method, the rinsing was only done prior to autoclaving. It was hypothesized that autoclaving disturbed the cell wall, thereby releasing organic matter that was within the cells. With no washing after autoclaving, the released matter remained with the biomass and in turn was released to the liquid samples. In addition, since the cells were live

when the washing occurred, it was their nature to try to equilibrate the external liquid concentration with the liquid in the cell (Oxford University Press, 2004). This could explain why it was difficult to achieve a wash water pH equivalent to that of distilled water. If the cells were rupturing due to this concentration difference, salts would have been released during biomass washing.

To determine why the "wash after autoclaving" method was less effective than the "double wash" method was challenging. In theory, the after autoclave wash should have remedied the issue of organic matter being released from the cells as a result of the autoclaving. However, other explanations are possible. For example, since the biomass was autoclaved in the medium, this could have allowed additional medium constituents to enter the cells as a result of the cell disruption due to autoclaving and, therefore, become difficult to remove during washing.

Overall the "double wash" method produced the "cleanest" biomass of the three wash methods tested and this approach was used from this point on.

Although the results of this Biomass Washing Study had potential to negate the data from the previous Optimum Pre-Treatment and Optimum pH Mini-Studies, the results from this Biomass Washing Study for colour showed no significant difference in the colour values after the addition of biomass into distilled-deionized water. For example for the "double wash" method the average colour value of the water was approximately 20 TCU whereas the "wash before autoclaving" method used in the previous studies had an average of approximately 40 TCU. Therefore the difference in colour was not significant in this study where colour removal values were in the hundreds of TCU range. Optimum pretreatment and pH was selected based on colour removal, not COD removal.

Interference with biosorption could have occured from the high COD levels found in the "wash before autoclaving" biomass washing method. However, although the Optimum Pre-Treatment and Optimum pH Mini-Studies were not repeated with "double wash" prepared biomass, it was felt that the previous test results would be indicative enough regardless of some interference from the COD. Additionally, the selection of the use of autoclave only and original pH made from these mini-studies were also based on practicality of process and not explicitly on the highest colour removal.

5.2.4 Mixing mini-study

In addition to the observance of excessive COD levels, there was also concern that mixing was not complete. To ensure that the batch testing was performed effectively, a study was carried out to analyze three variables: flask sizes, shaker, speed and dose, using Western Pulp effluent. From this test the filtered samples were analyzed for colour removal. The combination of mixing variables that provided the most colour removal indicated the most effective mixing.

Figure 7 shows results from runs 1 and 10 (125 rpm; 125 mL and 300 mL, respectively; 0.2 g) and runs 2 and 3 (125 rpm; 125 mL; 0.5 g and 0.8 g, respectively), and runs 11 and 12 (125 rpm; 300 mL; 0.5 g and 0.8 g, respectively), at 48 h, which compares the different flask sizes and biomass doses and the amount of colour that was removed. Additionally, Figure 7 also shows test results at 0.2 g (runs 2 and 3) with the biomass in contact with the effluent for 24 h, labeled "0.2 *ks test". These results are included since the results obtained from the mixing test at 0.2 g for 48 h contact were not consistent with other data obtained from the kinetic testing at 0.2 g. Since the data obtained in the kinetic study were more consistent with the trends of other results at dose rates 0.5 and 0.8 g, it was suspected that the data at 0.2 g from the mixing test was erroneous. As the kinetic study data for 0.2 g appears more appropriate, it was used for analysis.

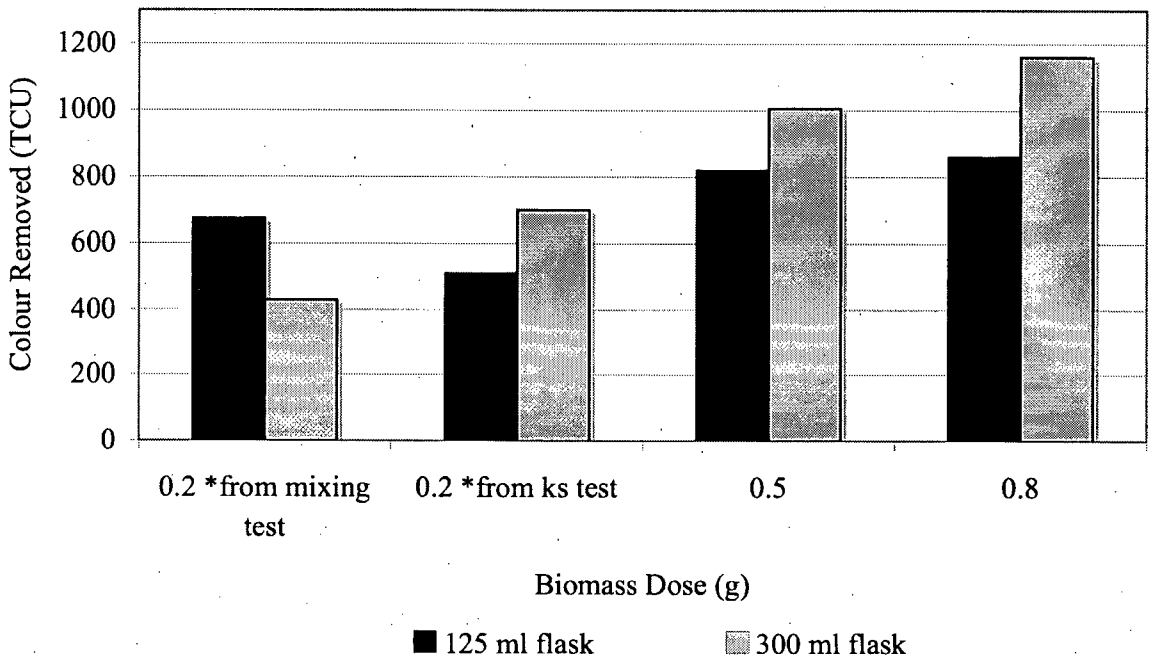

Figure 7. Mixing mini-study comparing the colour removal efficiency of the biomass with 48 h contact time using 125 mL and 300 mL flask sizes

Figure 7 shows that the test results indicated that the 300 mL flask size allowed for more mixing to occur than the 125 mL flask size, since colour removal was greater at 300 mL.

Figure 8, compares the results at different shaker speeds 125 rpm and 200 rpm, runs 10-12 (125 rpm; 300 mL; 0.2 g, 0.5 g and 0.8 g, respectively) and 16-18 (200 rpm; 300 mL; 0.2 g, 0.5 g and 0.8 g, respectively) on Western Pulp effluent with results of the total colour removed.

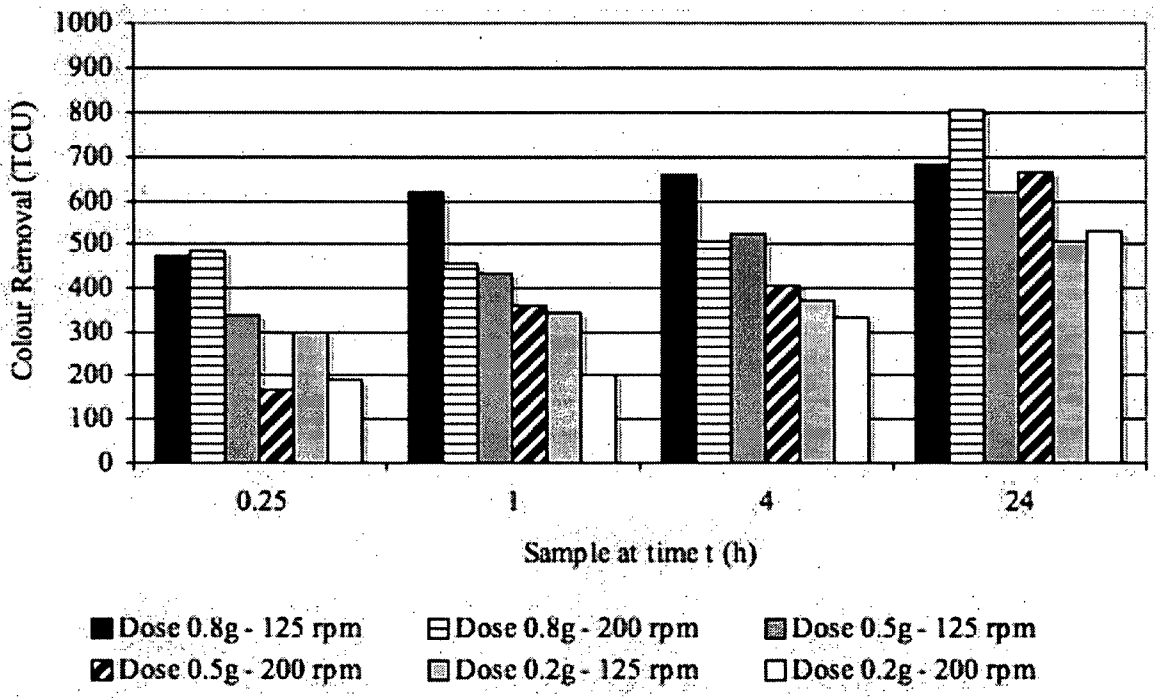

Figure 8. Mixing mini-study comparing the colour removal efficiency of the biomass with 48 h contact time using 125 rpm and 200 rpm shaker speed

The results for times 0.25, 1 and 4 h indicate that there was no improvement from the increased shaking speed since the amount of colour removed by the biomass did not show any consistent increase colour removal. However, the results at time 24 h showed a consistently greater colour removal, which indicated a benefit from increased shaker speed. It was likely that the increased speed did result in increased colour removal since the long-term removal at 24 h showed a consistent increase. Nonetheless, it was not completely clear that 200 rpm improved mixing, particularly due to the high level of error for colour measurement.

Observance of mixing at different flask sizes and rotation speeds showed that at 125 rpm, the 125 mL flask did not allow full suspension of all the biomass, particularly at the higher doses, 0.5 and 0.8 g. However, with the larger flask size, 300 mL, at 125 rpm all of the biomass was suspended. Further, both flask sizes at 200 rpm showed suspension at all doses. Therefore, at the time of research, it was assumed that increased shaking speed did not result in increased mixing. However, in retrospect, based on further analysis, this may not have been the correct assumption. The colour results shown in Figure 8 did not unquestionably indicate that the

increased speed from 125 rpm to 200 rpm was beneficial, however, the COD results for the same test run provides another perspective.

Figure 9 shows the total COD results for the mixing study for runs 10 -12 (125 rpm; 300 mL; 0.2 g, 0.5 g and 0.8 g, respectively) and 16 -18 (200 rpm; 300 mL; 0.2 g, 0.5 g and 0.8 g, respectively).

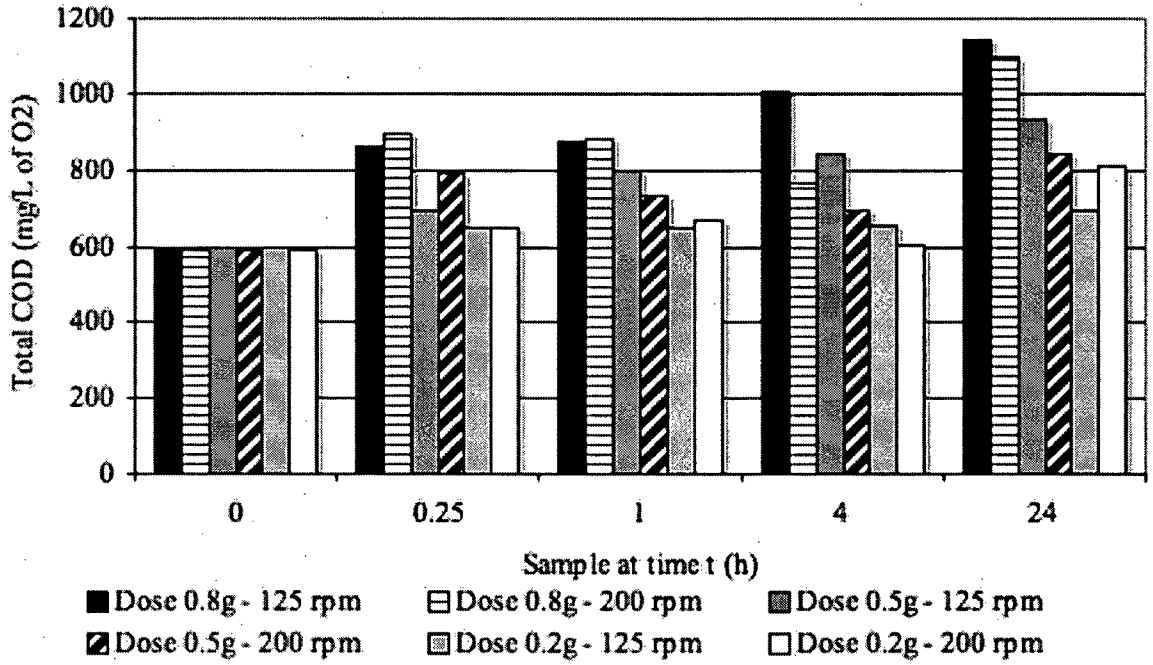

Figure 9. Total COD results of the sample from the mixing study using biomass doses of 0.2 g, 0.5 g and 0.8 g at 125 rpm and 200 rpm

Note the results shown for 0 h indicated the initial total COD of the effluent prior to biomass addition. The results show a definite improvement in total COD levels in the effluent with an increased shaker speed. Most of the runs showed higher levels of total COD, compared to the runs with the same dose, at 125 rpm than for 200 rpm. Since, from visual observance while testing, the fungus appeared suspended using a 300 mL flask size at both 125 rpm and 200 rpm, perhaps the increased shaker speed improved bulk solution transport, therefore enhancing the absorption. This could mean that the bulk solution transport was a limiting factor.

Finally, it appears that further testing of runs 14 and 15 and perhaps 13 (160 rpm; 300 mL; 0.5 g, 0.8 g and 0.2 g, respectively) should have been performed to clarify the results of this study. It is

likely that the increased shaking speed was increasing colour absorption at doses of 0.5 and 0.8 g. However, the results obtained for this study were not very clear. As well, mixing at 200 rpm was quite violent and since biomass production occurred at 125 rpm, maintaining the shaker speed at 125 rpm allowed biomass production and testing to occur simultaneously on the same shaker. Therefore, a shaker speed of 125 rpm was warranted.

5.2.5 Kinetic mini-study part 1.

The kinetic mini-study was performed to determine both the kinetic rate and the approximate equilibrium point of absorption. This was determined from comparison of colour removal efficiencies at different contact periods. Both effluents were utilized in this mini-study.

Figure 10 displays the amount of colour removed from Western Pulp and Howe Sound effluent through time with a biomass dose of 0.2 g.

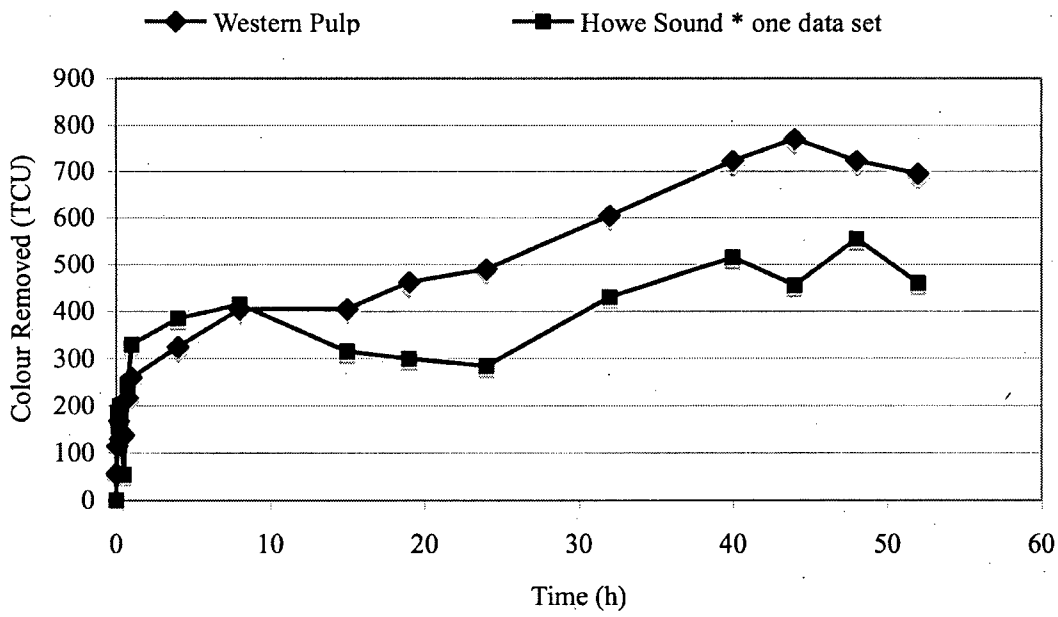

Figure 10. Kinetic study of Western Pulp and Howe Sound effluents at a dose of 0.2 g and time interval up to 52 h

With the initial observation, it appeared that there were two phases of removal, one that plateaued around time 8 to 15 h, and another that began around 19 to 32 h. This raised the question; what caused the removal for this second phase? This may have been a result of

chemical, biological or biochemical mechanisms. The appearance of a second phase of removal at such a time leads one to suspect that biological mechanisms are the cause. In response, testing was performed on the biomass under biologically-inhibited conditions.

5.2.6 Inhibition mini-study

The inhibition mini-study was used to determine if biological activity was causing this second phase of removal or if it was a result of other mechanisms. This was determined by comparing the colour removal efficiencies using three inhibition methods; a reduced temperature of 4°C, addition of NaN_3 and addition of NaF.

Figure 11 shows the results of various biologically inhibiting conditions or biological chemical inhibitors on Western Pulp effluent on the colour removal efficiency of the biomass.

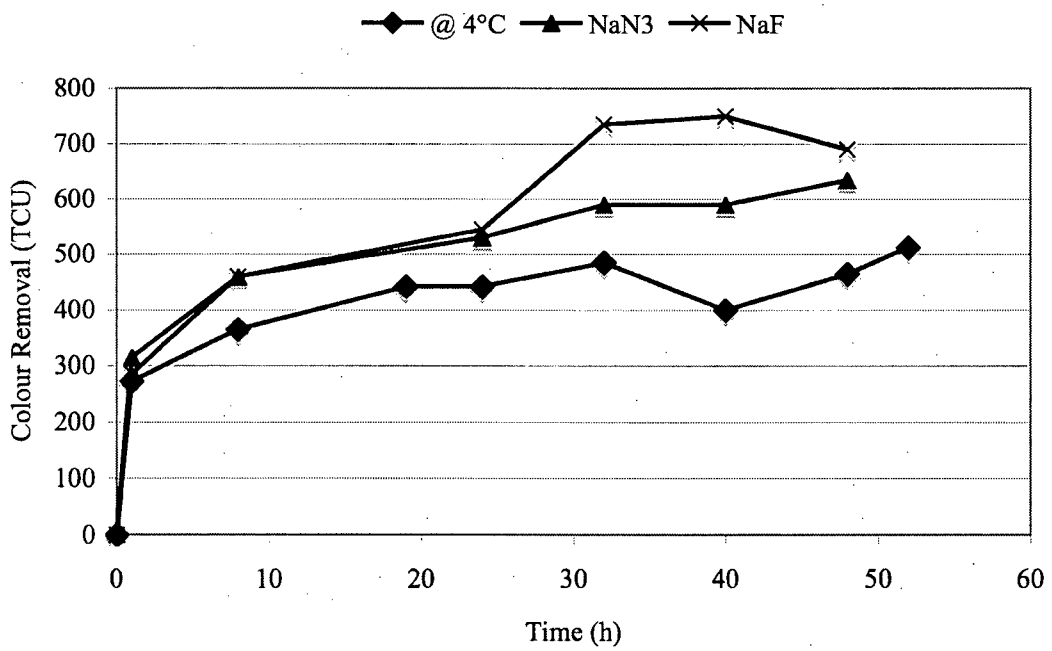

Figure 11. Biologically inhibited kinetic study of Western Pulp effluents at a dose of 0.2 g and time interval up to 52 h

Note that the results for NaN_3 and NaF testing were obtained from single runs, whereas the test runs at an ambient temperature of 4°C was run twice. Both the NaN_3 and temperature-inhibited treatments showed only a very small increase in colour removal at time 19 to 32 h. Therefore, it

was reasonable to assume that biological activity contributed to the delayed removal observed more predominately in the Kinetic Study Part 1 where no biological inhibitor was utilized. Therefore, in an effort to "clean up" the biosorption results to allow for a less complicated description of the biosorption mechanisms, NaN_3 and temperature inhibition were used in the kinetic rate mini-study.

A colour removal increase did seem to occur after time 24 h for the effluent that was inhibited with NaF. It could be hypothesized that NaF was unable to inhibit the biological activity that was occurring in the batch tests, however, it could also be hypothesized that the dose in which it was applied that was incorrect. The dose selected for this study may have been too low to be effective.

In addition, the results at 4°C compared to the NaN_3 at room temperature revealed an increased biosorption under the NaN_3 test conditions, which may have been a result of the NaN_3 being run at a room temperature (approximately 20°C). These results prompted the temperature mini-study. Further discussion regarding these differences will be presented below.

5.2.7 Kinetic mini-study part 2

The kinetic rate study was continued using two of the previously tested biological inhibitors; an experimental temperature of 4°C and NaN_3 addition. It was intended with the inhibition of biological activity, the biosorption kinetic rate analysis of would have reduced interference providing a more precise comparison to the Lagergren and Ho et al. equations.

Figures 12 and 13 illustrate the results of the inhibited kinetic studies of Western Pulp and Howe Sound effluents, respectively, of colour removed from the effluent at the specified time intervals under the two biologically inhibited conditions.

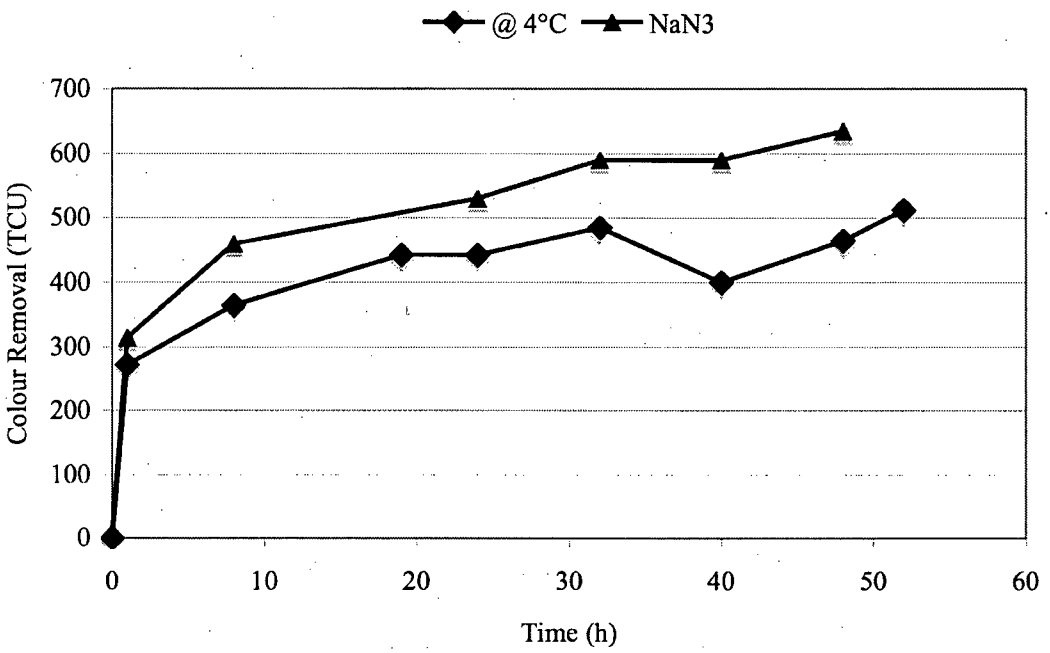

Figure 12. Biosorption of colour from Western Pulp effluent at various specified time intervals at 4°C and with addition of NaN_3

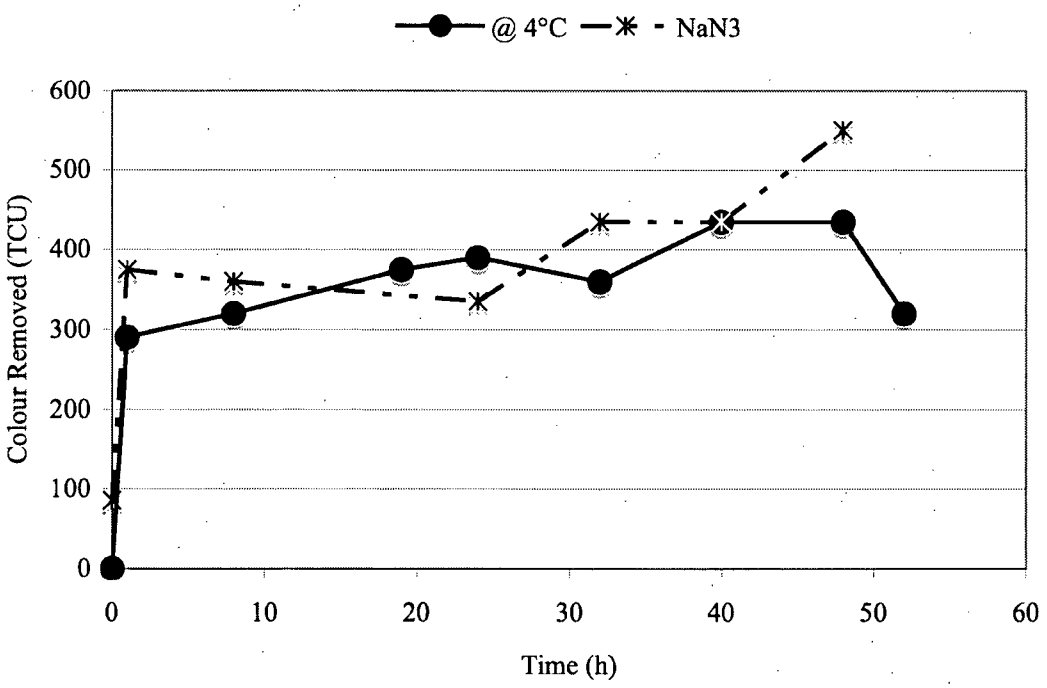

Figure 13. Biosorption of colour from Howe Sound effluent at various specified time intervals at 4°C and with addition of NaN_3

The results of colour removal due to biosorption using Western Pulp effluent, shown in Figure 12, indicated that removal of colour occurred significantly within the first 8 h of biosorption, with emphasis on the first hour, then continued slowly until measurement ceased at 48 h. The colour removal data from biosorption of Howe Sound effluent, shown in Figure 18, on the other hand, indicated more immediate removal, with most of the colour removal occurring in the first hour. After that point, biosorption reached a plateau, with only a slight further increase through time. The results of biosorption of colour using Howe Sound effluent appeared to have more variability and this could have been a result of the difficulty experienced while filtering the effluent. The filtering of the Howe Sound effluent with or without addition of the biomass was always more difficult than for the Western Pulp effluent. It was hypothesized that this is a result of the higher electrical conductivity levels in the Howe Sound effluent as seen in the Effluent Characterization Study results.

The results obtained from this mini-study were similar to other studies using dead biomass for biosorption in which removal took anywhere from 1 to 48 h to reach equilibrium, depending on initial effluent pH for dead *A. niger* used for the removal of metals, dyes and phenol (Fu and Viraraghavan, 2000; Fu and Viraraghavan, 2001b; Fu and Viraraghavan, 2002; Kapoor et al., 1999; Rao, 2001). However, in some studies, equilibrium took 3 days or 4 weeks to achieve as in the case of studies done by Zhou and Banks (1993), and Gallagher et al. (1992). As well, maximum removal periods vary; although, some have been reported to be as rapid as the first 10 min of contact (Banat et al., 1996).

To determine if the rate of biosorption fit either the Lagergren or Ho et al. equations, the data were analyzed using STATISTICA© version 6.1 statistical analysis software. Using a Gaussian-Newton estimation method and a 95% confidence level, the following results were obtained. Please note that 1 TCU = 1 mg of Pt-Co/L and this unit was used to describe the concentration of colour removal.

Table 15 presents the kinetic constants estimated for the Lagergren and Ho et al. equations by non-linear regression analysis of the measured data and, also, the statistical comparison of the resulting equation with the measured data.

Table 15. Results of non-linear estimation for colour removal for Lagergren *K* and Ho et al. *k*, biosorption rate models, using STATISTICA©

Effluent		Lagergren			Ho et al.		
		q_e (mg/g)	K (1/h)	R	q_e (mg/g)	k (g/mg·h)	R
Western Pulp 4°C	N=9	158.68	45.77	0.89	158.68	345.14	0.89
	Std. error	10.14	n/a		12	7686233	
	t (7)	n/a	n/a		13.71	0.00004	
	p-level	n/a	n/a		0.000003#	1.0	
Western Pulp NaN_3	N=7	195.02	53.08	0.87	195.02	435.92	0.87
	Std. error	17.97	n/a		21	19157976	
	t (5)	n/a	n/a		9.40	0.000023	
	p-level	n/a	n/a		0.000230#	1.0	
Howe Sound 4°C	N=9	137.11	50.96	0.92	137.11	294.59	0.92
	Std. error	7.13	n/a		8	3896835	
	t (7)	n/a	n/a		17.08	0.00008	
	p-level	n/a	n/a		0.000001#	1.0	
Howe Sound NaN_3*	N=7	155.63	51.51	0.91	155.63	351.60	0.91
	Std. error	11.87	n/a		14	8237917	
	t (5)	n/a	n/a		11.34	0.00004	
	p-level	n/a	n/a		0.000093#	1.0	

* note: extremely high results for t = 0 were thrown out and replaced by qt = 0 at t = 0
# this indicates that the model parameters estimated were statistically significant (t-test) at a 95% confidence level

For the Lagergren model, the q_e and K estimated by non-linear regression using STATISTICA© indicated that the values were degenerated and therefore may not be correct. Therefore, the statistical values for t-test, p-level and standard error for K are not applicable. The estimated values of k obtained for the Ho et al. model were statistically significant at a confidence level of 95% for q_e but not for k. This indicates that 95% of the measured data fit the Ho et al. equation with the q_o values generated by STATISTICA©, however, the estimated k did not fit with 95% of the measured values. The p-level indicates the probability of the relationship of the variable as being a "fluke", i.e. indicating the statistical significance of the relationship (StatSoft Inc., 2006). Therefore the lower the p-level the less likely the measured data's fit with the model is a "fluke".

Nonetheless, based on the R values, the correlation co-efficient, it can be said that correlation of the measured data to the Lagergren and Ho et al. model, in general, was good. The range of possible R values is between 0 and 1, therefore values between 0.87 and 0.92 show good

correlation. The model could be used to predict the kinetic rates of colour biosorption of fungal biomass on the treated whole mill pulp effluents used in this study.

Figures 14 to 17 show the comparison of the measured kinetic rate data obtained from the Kinetic Mini-Study Part 2 with the Lagergren and Ho et al. equations with constants estimated by the non-linear estimation of the STATISTICA program, using two different biological inhibitors on both the Western Pulp and Howe Sound effluents. The constants obtained from non-linear estimation based on the Lagergren and Ho et al. models provided nearly identical curves for the intervals over which the data were measured and therefore overlap in the Figures 14 to 17.

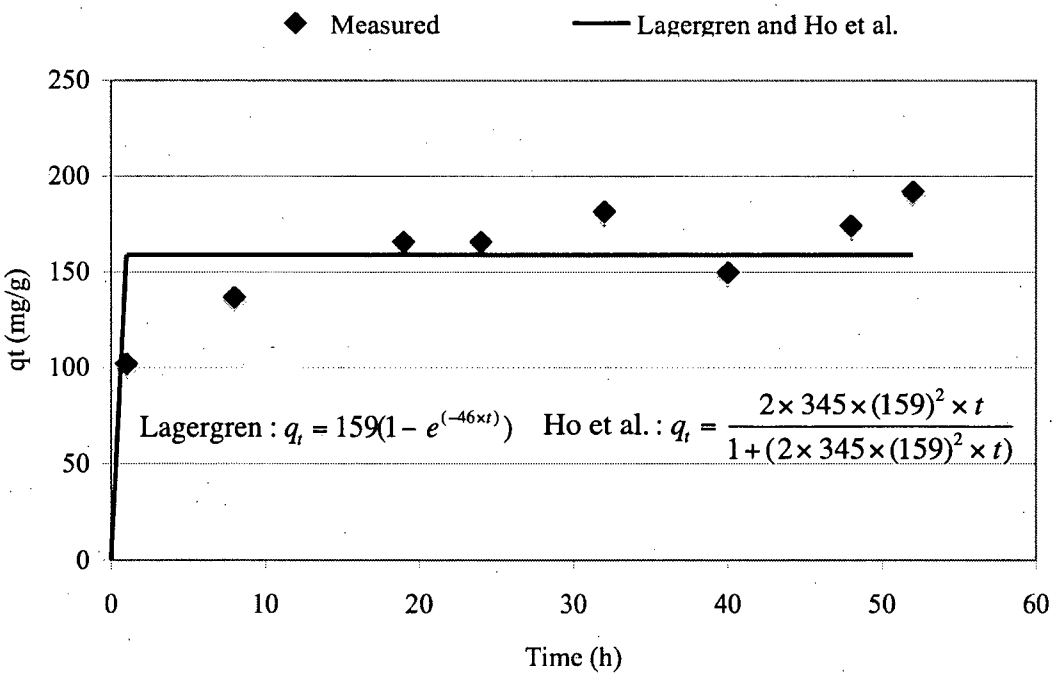

Figure 14. Kinetic Study: Lagergren, Ho et al. and measured data - Western Pulp effluent at 4°C

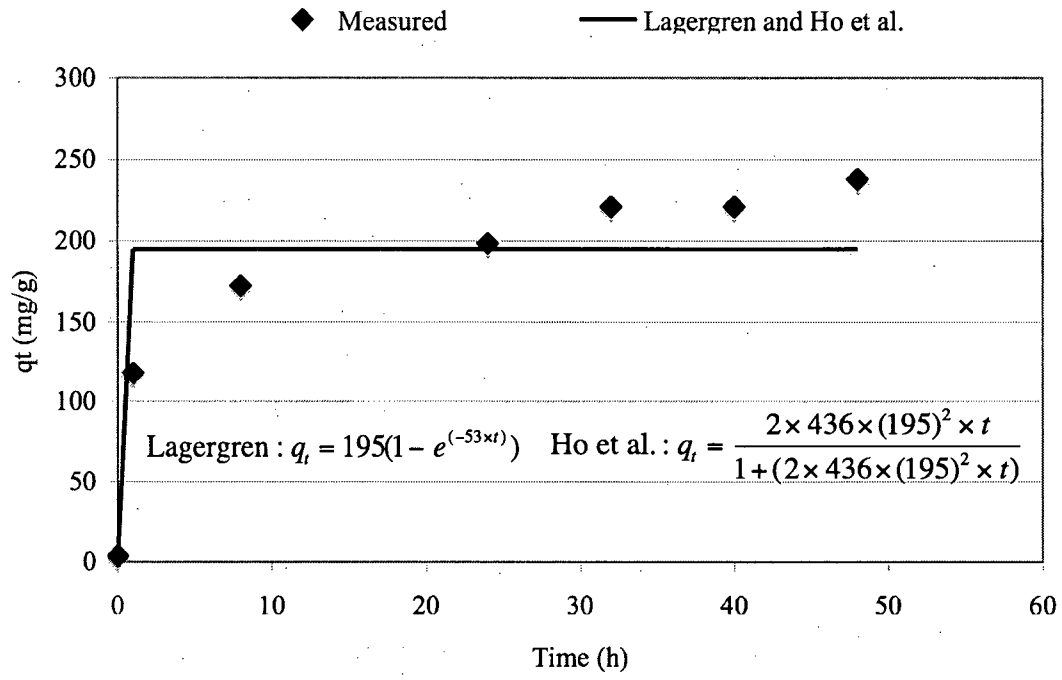

Figure 15. Kinetic Study: Lagergren, Ho et al. and measured data - Western Pulp effluent with NaN_3

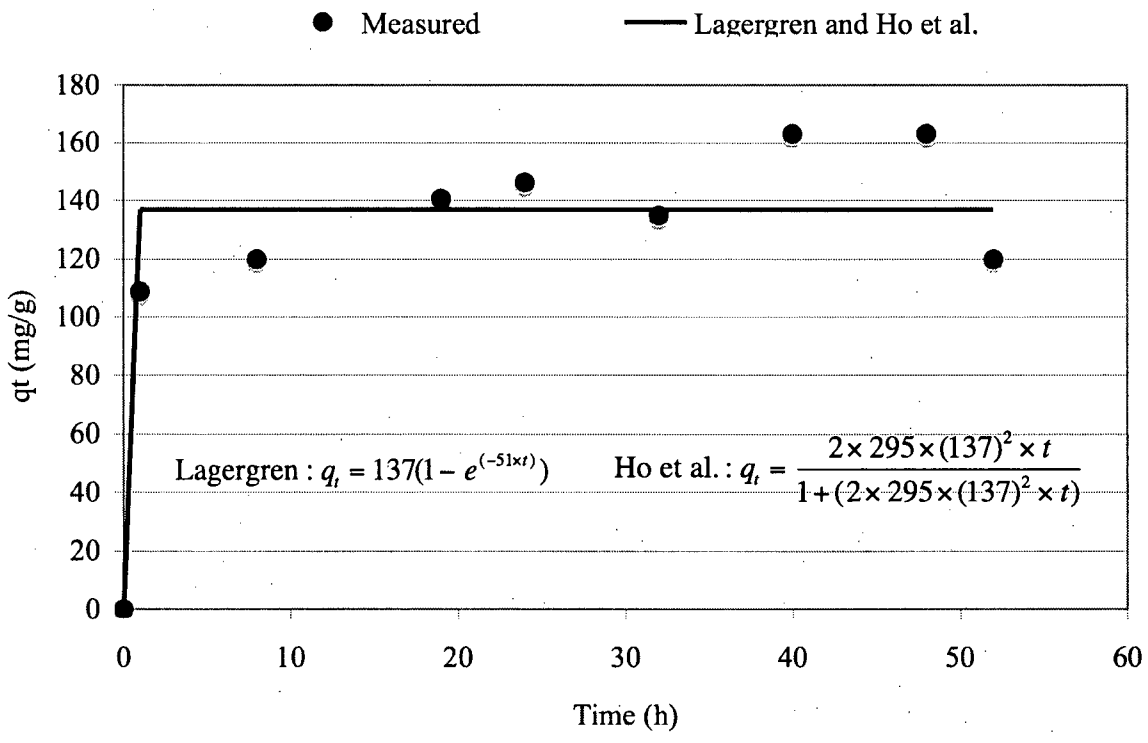

Figure 16. Kinetic Study: Lagergren, Ho et al. and measured data – Howe Sound 4°C

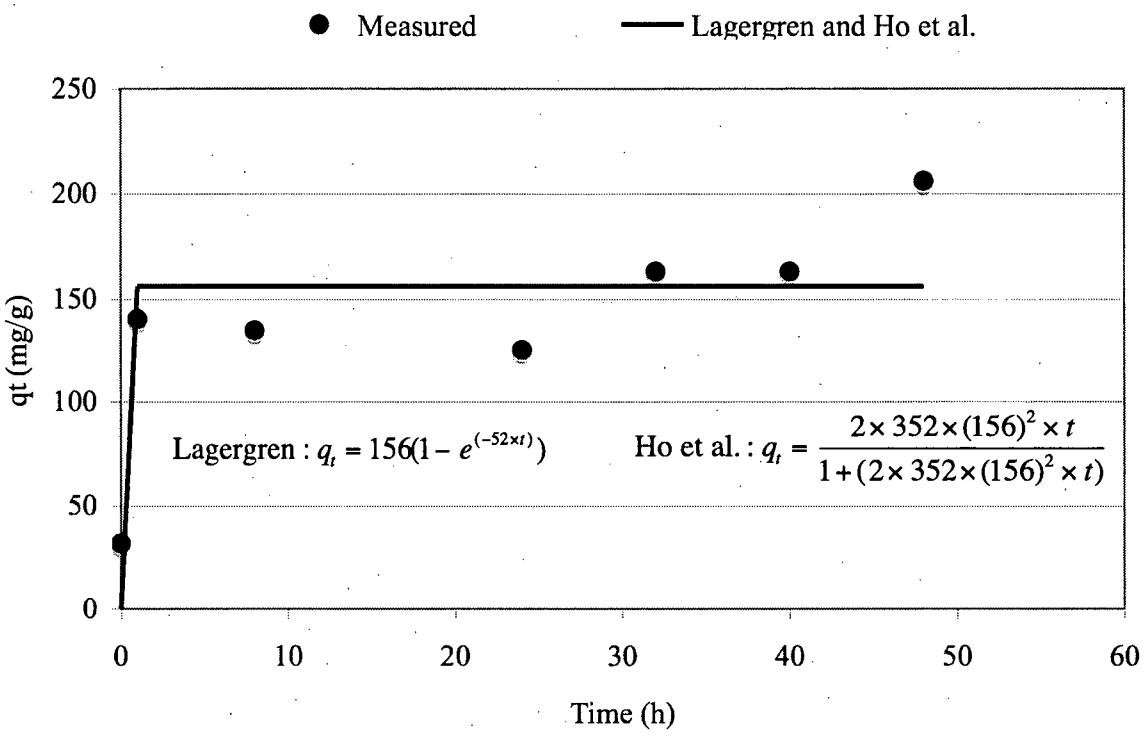

Figure 17. Kinetic Study: Lagergren, Ho et al. and measured data – Howe Sound NaN_3

5.2.8 Isotherm mini-study

The results of the isotherm mini-study were very informative as they gave an indication of the biosorption mechanisms of the biomass for removal of colour. The isotherm mini-studies were conducted on both effluents with addition of NaN_3 as an inhibitor. Colour removal was measured and used to determine equilibrium dose and the best fit isotherm model, either the Langmuir, Freundlich or BET.

Figures 18 and 19 show the colour removed by biosorption obtained in the isotherm testing using different doses of biomass.

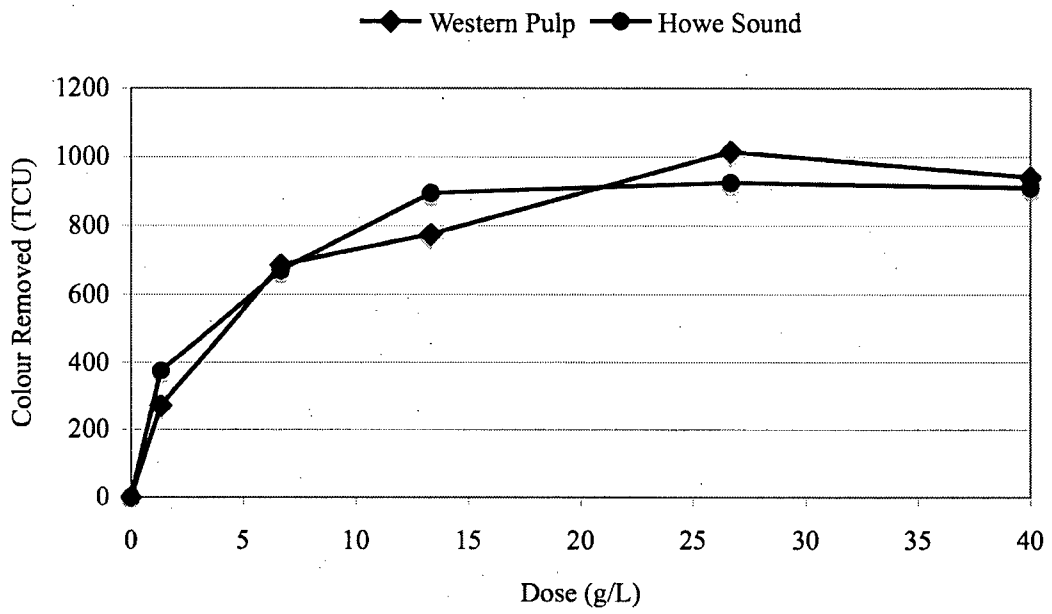

Figure 18. Isotherm Study: Colour removal of both effluents with NaN_3 addition at 32 h at room temperature

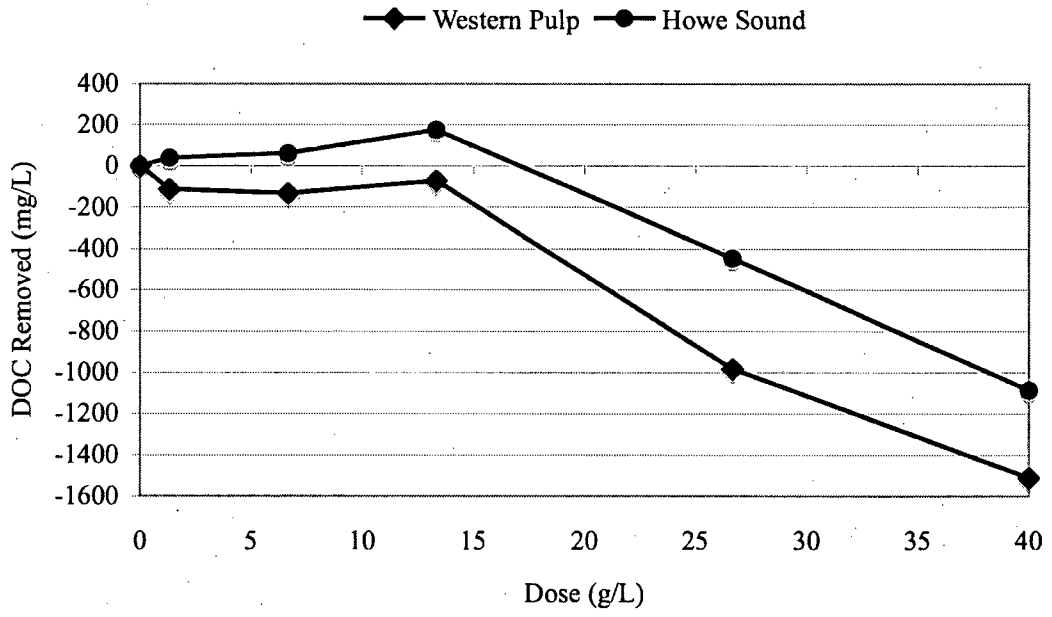

Figure 19. Isotherm Study: DOC removal of both effluents with NaN_3 addition at 32 h at room temperature

The test results for colour removal showed the maximum removal values for each effluent at approximately 900 TCU, which is a significant amount of colour removed. This reflects a removal of approximately 55% of the Western Pulp and 75% of the Howe Sound effluent colour. This is roughly a moderate-to-good removal efficiency compared to other removal colour removal technologies available.

The test results for DOC removal indicated that when applying the biomass to the Howe Sound effluent, DOC removal was possible, up to approximately 200 mg/L. However, above a certain dose of biomass, the amount of DOC introduced by the addition of the biomass was greater than that removed. The DOC removal of the biomass on the Western Pulp effluent did not provide enough removal at any point that was able to counter the amount of DOC introduced by the biomass.

It was difficult to explain the difference of addition of organics between the two effluents after treatment since colour removal for Western Pulp effluent was very similar to Howe Sound effluent. It would be more logical to see a consistent removal of constituents from each effluent. One explanation of the difference in organics loading may be a result of differing chemical strengths of the effluents.

In order to better understand the type of adsorption that was occurring, non-linear estimation of the Langmuir, Freundlich and BET constants was performed and then correlation of the resulting equations were applied to the measured data. Table 16 indicates the correlation of the measured data to these models. Again, STATISTICA© version 6.1 statistical analysis software was used, employing the Gaussian-Newton estimation method and a 95% confidence level.

Table 16. Isotherm mini-study results compared to Langmuir, Freundlich and BET models

Effluent		Langmuir			Freundlich			BET		
		Q^o (mg/g)	B	R	K_F	N	R	Q^o (mg/g)	B	R
Western Pulp	N=6	84.99	1350	0.47	n/a	n/a	0	36.75	3.59	0.97
	Std. error	126.92	$2x10^9$		n/a	n/a		4.74	3.98	
	t (4)	0.0000	0.6696		n/a	n/a		7.75	0.90	
	p-level	1.00	0.5398		n/a	n/a		0.0015*	0.418	
Howe Sound	N=6	101.20	487.83	0.40	n/a	n/a	0	126.64	0.99	0.99
	Std. error	141	120377653		n/a	n/a		19.34	0.40	
	t (4)	0.718	0.0000		n/a	n/a		6.55	2.51	
	p-level	0.5124	1.0		n/a	n/a		0.0028*	0.0663	

* this indicates that the model parameters estimated were statistically significant (t-test) at a 95% confidence level

Of the estimated data listed in Table 16, only values for Q^o obtained from the BET model were statistically significant at a 95% confidence level; the estimated constant was verified to be correct with a 95% of the measured data. Estimation of the constants of the Freundlich equation was not possible, since, the measured data could not fit the Freundlich equation.

Based on the R values of the Langmuir and BET equations, the correlation of the measured data with these models, in general, is sufficient for prediction of biomass dose rates. The BET model at R values of 0.97 and 0.99 for Western Pulp and Howe Sound effluent, respectively, shows a superior correlation than the Langmuir equation at 0.47 and 0.40 for Western Pulp and Howe Sound effluent, respectively. The BET model applies the Langmuir model to each monolayer and, therefore, it was logical that the Langmuir applied poorly yet the BET model applied so well. The excellent correlation with the BET model confirmed the assumption that biosorption with dead *A. niger* biomass was a physical process. This also led to the conclusion that multi-layer adsorption was occurring.

Figures 20 and 21 show the measured isotherm data, inhibited by NaN_3, and the BET equation using the constants estimated with non-liner regression by STATISTICA©.

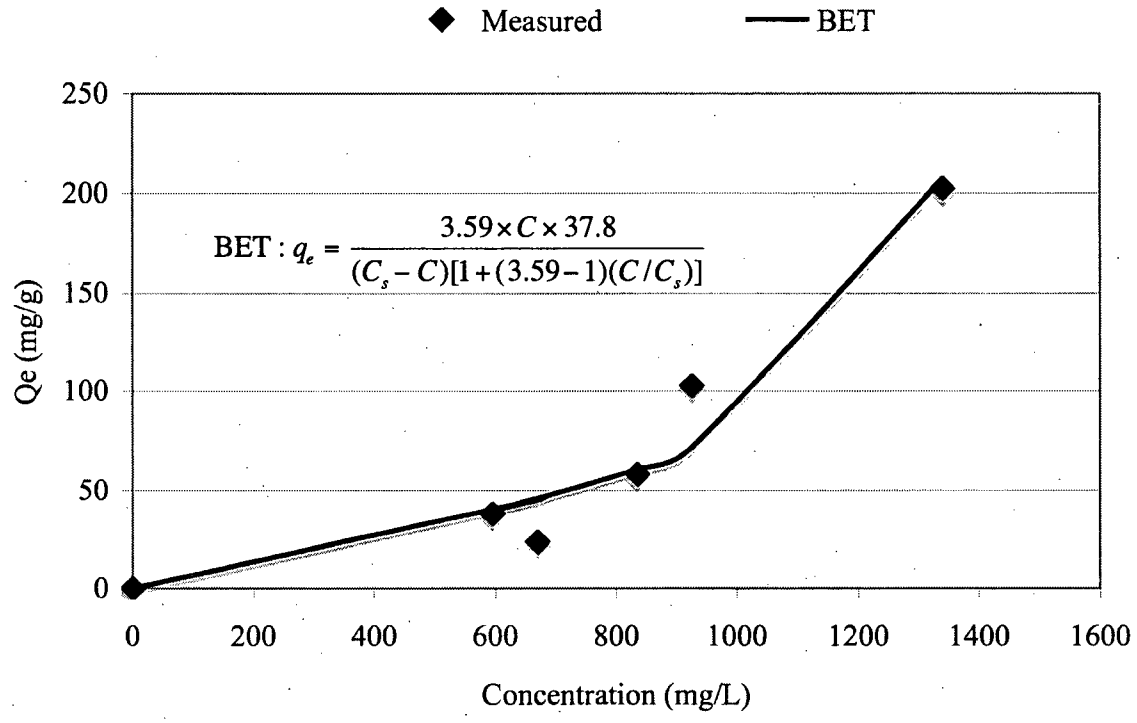

Figure 20. Kinetic Study: BET and measured data - Western Pulp effluent with NaN_3 at room temperature

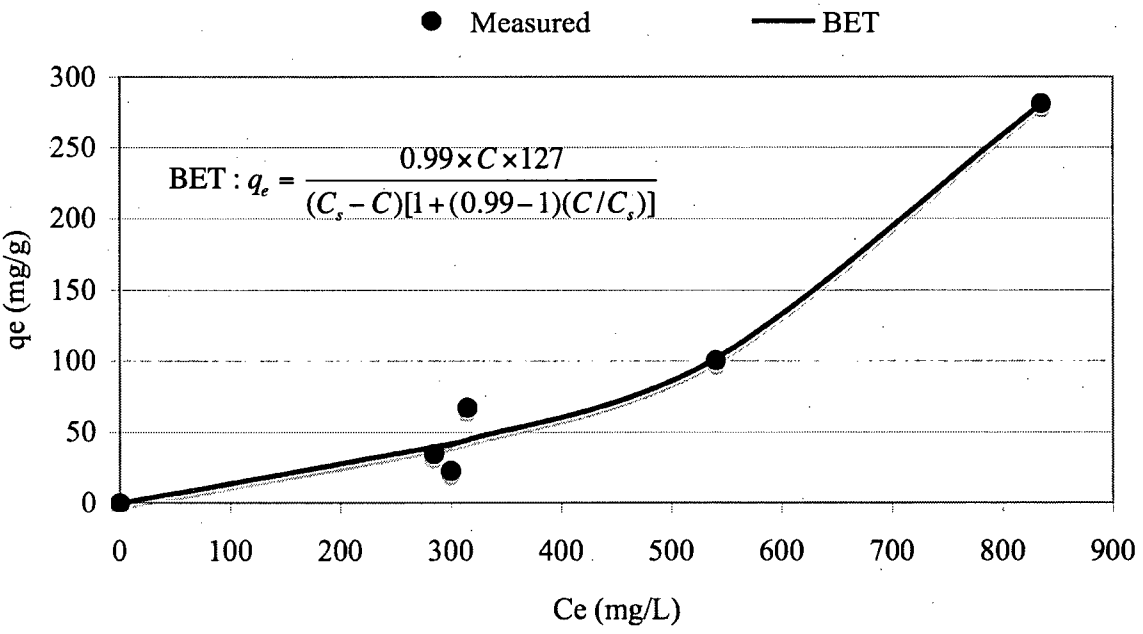

Figure 21. Kinetic Study: BET and measured data – Howe Sound effluent with NaN_3 at room temperature

5.2.8 Removed fraction mini-study

Isotherm Mini-Study samples at a dose of 1.0 gram and the experimental blank, for both effluents, were analyzed by molecular weight fractionation in order to identify the fractions removed by biosorption.

Figures 22 and 23 illustrate the total colour and TOC, respectively, in each fraction of the samples of dose 0 g and 1.0 g for the Western Pulp and Howe Sound effluents.

Generally, colour removal occurred in all the molecular weight fractions of both effluents except for those fractions below 500 Da. The data in Figure 22 also indicated that colour in the <500 Da fraction increased slightly after contact with the biomass. It was difficult to determine if this was due to test method error or to a true increase. Notably, most of the colour removal from the Western Pulp effluent occurred in the 10,000 to 100,000 Dalton range, whereas with the Howe Sound effluent, colour was removed in the 500 to 3,000 Da and the 10,000 to 100,000 Da ranges. This lower molecular weight colour of 500 to 3,000 Da was not present in the experiment blank of the Western Pulp effluent, meaning there was no colour fraction in the effluent to be removed. Therefore, it was concluded that dead *A. niger* biomass removed the medium-to-high molecular weight fractions of colour but was ineffective for very low, less than 500 Da, and very high, greater than 100,000 Da, fractions. The medium-to-high molecular weight fractions have also been reported to have been removed in colour treatment processes such as lime treatment (Dugal et al., 1974).

The TOC data in Figure 23 also revealed that organics were being removed by the biomass, however, an increase of organics in a different fraction was resulting. Again removal seemed to be occurring in the mid-range of molecular fractions as seen with colour. However, the increase in very high molecular weight fractions was significant. This occurred more dramatically for the Western Pulp effluent, which confirmed previous results indicating post biosorption COD levels were higher than those before biosorption, ie. after the addition of the biomass to the effluent. This also occurred for the Howe Sound effluent to a smaller extent, which was not as noticeable since it was masked by organic removal of other fractions. These results support the results of the Biomass Washing Mini-Study where organics were being introduced to the sample fluids, the treated plant effluent or distilled-deionized water, from the addition of the biomass. Nonetheless, the biomass was still effective at colour removal. Biosorption of Howe Sound effluent was still

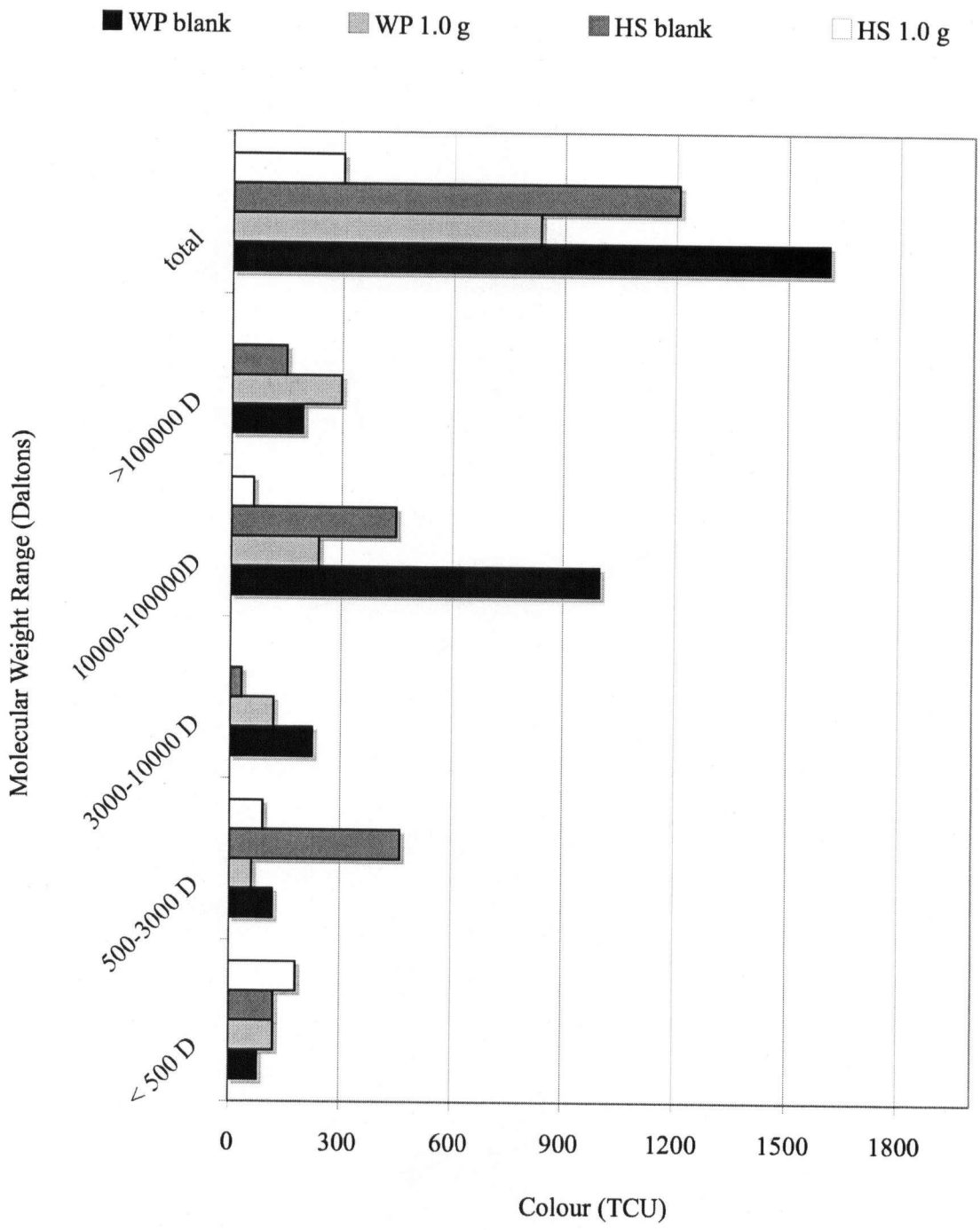

Figure 22. Molecular weight distribution of colour for both effluents after treatment

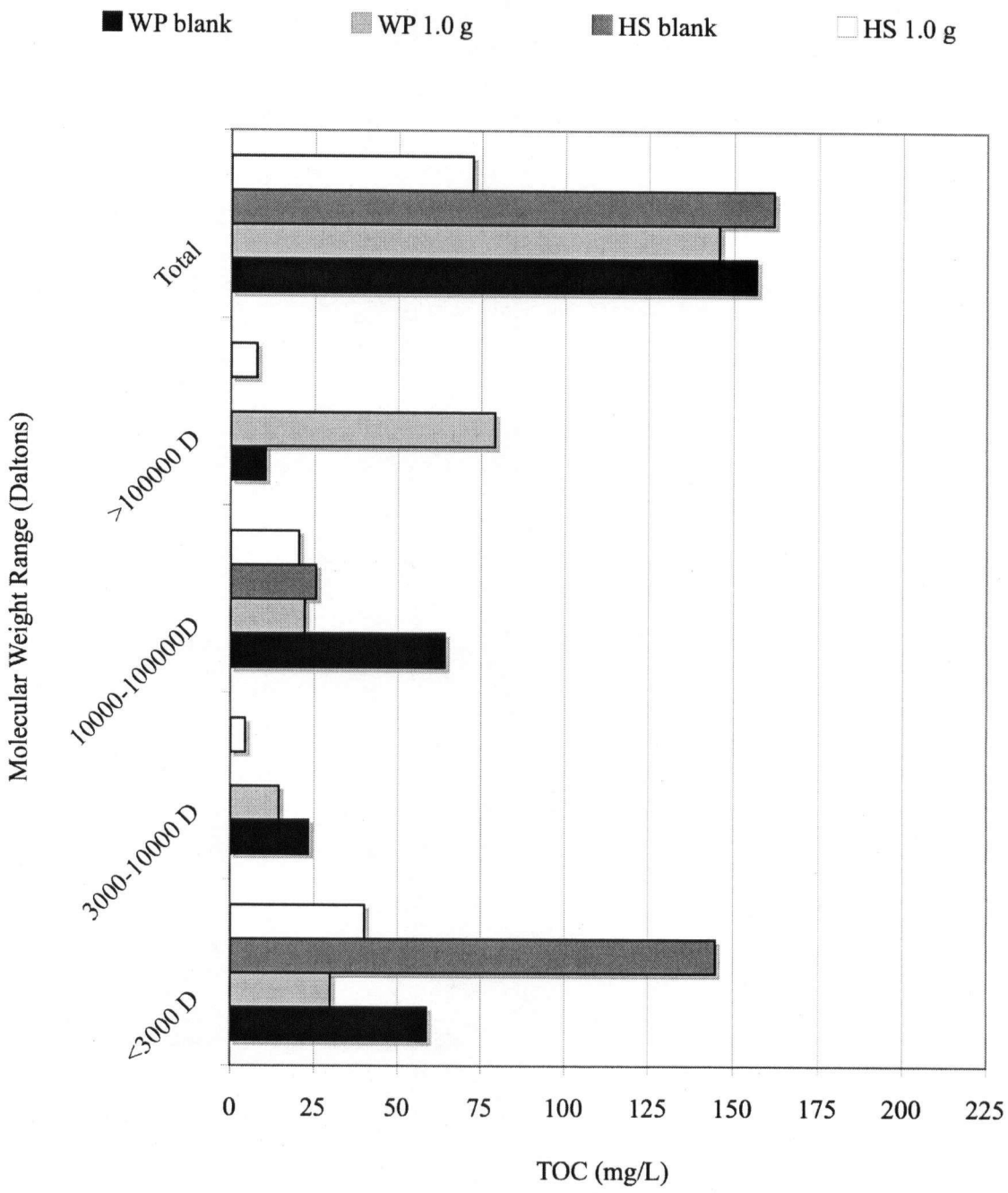

Figure 23. Molecular weight distribution of TOC for both effluents after treatment

able to show an overall removal of organics when biomass doses were not excessive, ie. the doses where additional biomass provided no further colour removal. As there was organic removal occurring by the fungus it would be interesting to research if other problematic pulp mill

effluent constituents, such as those measured as absorbable chlorinated organics, AOX, is removed with these organics.

5.2.9 Temperature mini-study

From the previous kinetic data it was observed that temperature affected the rate of color removal. Therefore, a test run was performed at 35°C with the addition of the NaN_3 inhibitor to identify the effects of temperature.

Figure 24 shows the total colour removed with an experimental temperature at 35^0C with NaN_3 addition and the previous results of Western Pulp effluent at 4°C without NaN_3 and 20°C with NaN_3.

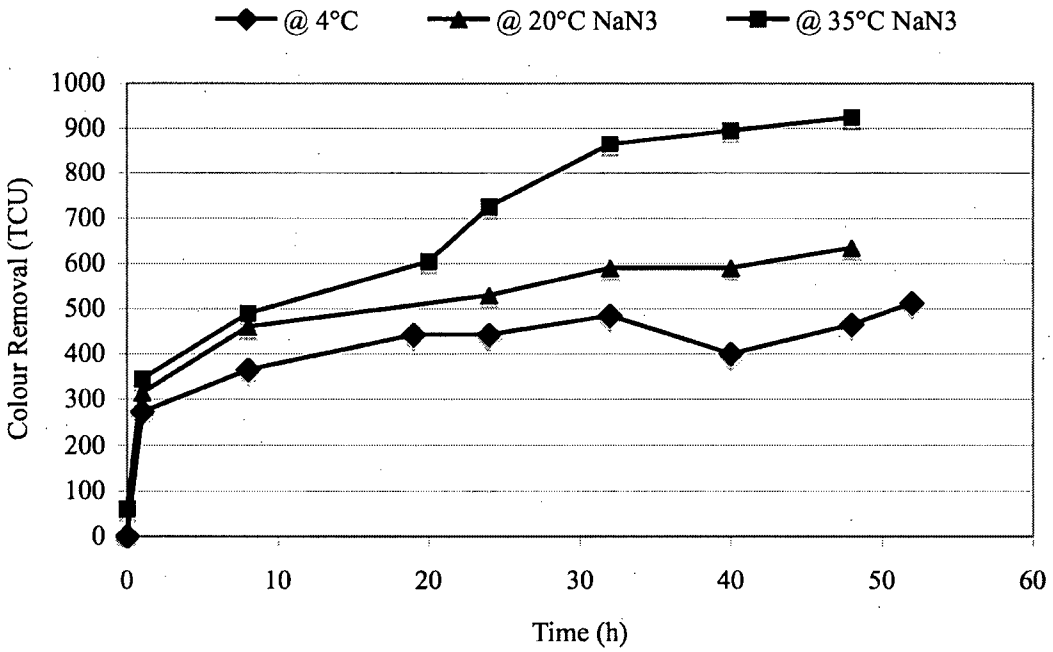

Figure 24. Colour removal at different temperatures

Unfortunately, it appears that the inhibitor dose of 0.1 mM was not enough to prevent biological activity at the higher temperature of 35°C. However, it was evident throughout time and prior to 20 h the removal ability at 35°C was greater than removal at lower temperatures. This was unexpected since it was assumed that biosorption with dead *A. niger* was a physical phenomenon. Physical adsorption should decrease as temperature increases (International Union

of Pure and Applied Chemistry, 1971). Even though this was contrary to the principles of physical adsorption, this does not imply that physical adsorption was not the principal biosorption mechanism. Instead, this could indicate that chemical mechanisms were limiting factors in the biosorption of pulp mill effluent colour to dead *A. niger* biomass.

In the mixing study it was observed that although the biomass was suspended, more rapid mixing might have increased biosorption. In combination with the results of this mini-study, in which temperature increased biosorption, the results of the mixing mini-study may have indicated that the physical process of adsorption was readily occurring but the transport of the colour to the adsorption sites was limiting. As noted in Section 2.4.1 Basics of Adsorption, there is a series of steps that occurs prior to the absorbate reaching the adsorption site; bulk liquid transport, the absorbate being moved to the vicinity of the stagnant liquid film, and diffusion of the absorbate through the stagnant film layer. Mixing impacts the bulk liquid transport and diffusion is based in chemistry, which is influenced by temperature. Therefore the temperature effect could be a result of the increased diffusion of the adsorbate.

5.3 Practical Application Study

The application of biomass in a simulated real world treatment system was assessed to determine the feasibility of the practical use of dead *A. niger* biomass. This study was performed at the Howe Sound pulp and paper mill on February 22, 2006. Batch testing was performed that mimicked the treatment process the plant used, using effluent and return activated sludge samples taken from the transfer pipes to the aeration tank.

Table 17 details the colour, pH, TSS, TOC and BOD measurements of the five batch test run, Blanks 1 and 2 and a dose of 0.8 g, 4.0 g and 8.0 g in 300 mL of mixed liquor. In Table 17, the Blank sample indicates the average of experimental Blanks 1 and 2 where no biomass was added. Figure 25 shows the total colour removed at each dose.

Table 17. Practical application of study results

Dose (g)	Dose (g/L)	Colour (TCU)	pH	TSS (mg/L)	TOC (mg/L)	BOD (mg/L)
Blank	0	995	8.45	23	113	43
0.8	2.67	595	8.14	20	123	118
4.0	13.33	440	7.82	47	185	730
8.0	26.67	340	7.75	220	>189	>1000

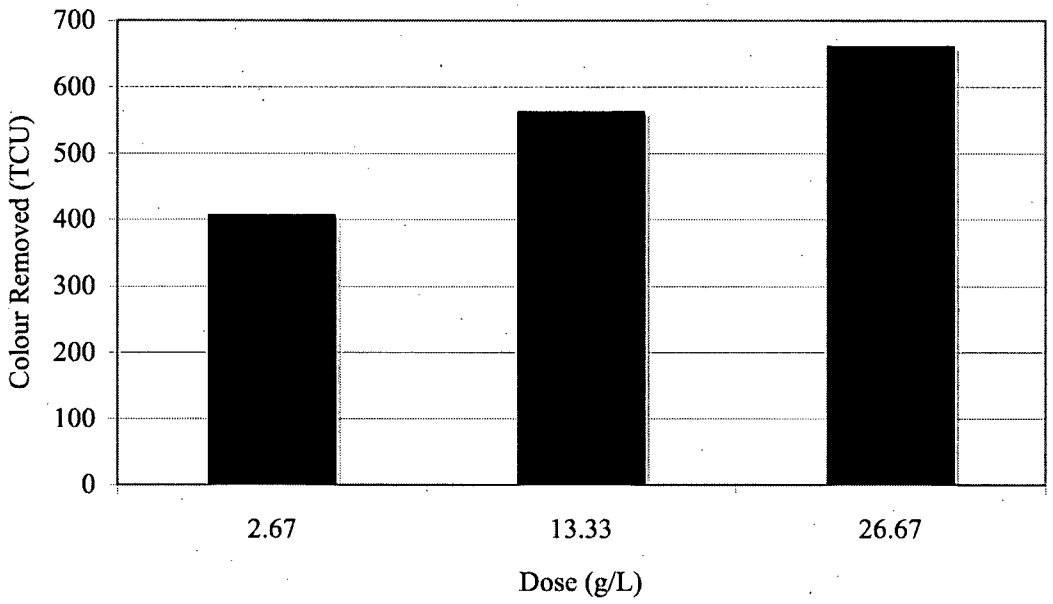

Figure 25. Colour removal in practical application study samples using three biomass dose rates

The application of the biomass in a batch activated sludge system did result in colour removal that increased with an increase in dose. However, the purpose of this study was only to determine if colour could be removed in a real world application and, therefore, no optimization of this process was studied. Therefore, it was concluded that with further research there is potential for use of dead *A. niger* biomass, in powdered form, in an activated sludge process.

6. CONCLUSIONS AND FURTHER RESEARCH RECOMMENDATIONS

Based on the research performed, the following conclusions have been made.

1. Based on colour removal efficiencies, autoclave only pretreatment was the most effective for measurement of colour at 400 nm. Autoclave only pretreatment was also selected based on its ease in preparation.

2. Initial effluent pH did have an effect on the biosorption rate and also the solubility of chromophores. In terms of colour removed by biosorption only, pH 4 followed by pH 8 showed the greatest removal.

3. Biomass washing and the mixing of the biomass and effluent did affect colour and organic removal efficiencies.

4. At approximately 24 h of biosorption, a second phase of removal occurred. This second phase did not occur when the effluent was inhibited by temperature or chemicals and therefore was contributed to biological activity.

5. The kinetic rate study revealed that colour removal by biosorption occurred most readily in the first 8 h with Western Pulp effluent, and within the first hour for Howe Sound effluent. Lagergren and Ho et al. models can be used to describe the kinetic removal rates, however, the correlation was reasonable but not exceptional.

6. For both effluents the maximum colour removal was over 900 TCU, with a biomass dose of about 20 g/L and 13 g/L for Western Pulp and Howe Sounds effluent, respectively. The isotherm study data fit the BET isotherm model very well and the Langmuir isotherm model moderately. This indicated that adsorption was occurring in a multi-layer fashion.

7. It was found that the biomass removed medium to high molecular weight molecules between 500-100,000 Daltons.

8. Temperature increase also increased biosorption of colour in the Western Pulp effluent between 4 and 35°C.

9. Based on data from the pretreatment, pH, kinetic rate, isotherm and removed fraction mini-studies, it was concluded that physical adsorption was the main mechanism used in biosorption of effluent colour on autoclaved *A. niger* biomass. In addition, the limiting factors in this study appeared to be bulk solution transport, and stagnant film diffusion.

10. There is potential for use of dead fungal biomass in activated sludge systems. Application of biomass in an activated sludge system did show colour removal from the effluent but did impact the TSS, TOC, and BOD removal efficiency. No further testing was done to improve this process.

As well, the follow areas could be researched further:

1. Limiting factors that affect biosorption. With the data obtained in the mixing and temperature mini-studies it was observed that bulk solution transport and stagnant layer diffusion was limiting.

2. The current study found that organic matter was also removed by the biomass. Research to identify this organic matter could reveal further benefits of *A. niger* biosorption. For example, more toxic organic constituents such as those measured as AOX, may be biosorbed.

3. The current study showed organics being introduced by the biomass into the effluent. It would be important to determine if other pretreatment forms could counter this organic addition yet still provide good colour removal.

4. As it was found that biosorption with *A. niger* could remove colour within an activated sludge system, further studies could be performed to determine if biosorption could occur without upsetting the activated sludge system.

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APPENDIX 1. CALCULATIONS

Colour

Colour values are determined through absorbance measurement of standard values and extrapolated accordingly. The following is a work example of colour value determination. Table A.1 lists the values used.

Table A.1 Worked example values

Sample	Absorbance
Blank	0
Std. 50	0.024
Std. 100	0.039
Std. 300	0.082
Std. 500	0.143
500 Daltons	0.027

Using Microsoft® Excel® 2004 a linear equation was determined using the standard values. For the values of obtain in Table A.1 the Equation A.1 was found.

$$y = 0.0003x + 0.0063 \qquad \text{(A.1)}$$

Where x represents the absorbance value and y is the colour value (TCU). This equation has a R^2=0.9905 and provides a y value of 78. This value is then multiplied by the dilution ratio, which in this example is 5, and provided a final colour value of 389 TCU for a x value of 0.027.

COD

COD values are determined through absorbance measurement of standard values and extrapolated accordingly. The following is a worked example of COD value determination. Table A.2 lists the values used.

Table A.2 Worked example values

Sample	Absorbance			Value	Average
Blank	0			0	
Std. 100	0. 036			0. 036	
Std. 300	0.114			0.114	
Std. 500	0.197			0.197	
Std. 800	0.317			0.317	
HCl	0.146	0.145	0.145	0.145	
HCl	0.141	0.141		0.141	
HCl	0.137	0.137		0.137	
HCl	0.134	0.134		0.134	0.139

Using Microsoft® Excel® 2004 a linear equation was determined using the standard values. For the values of obtain in Table A.2 the Equation A.2 was found.

$$y = 0.0003x + 0.0063 \quad \text{(A2)}$$

Where x represents the absorbance value and y is the COD value (mg/L). This equation has a R^2=0.9997 and provides a y value of 356 for a x value of 0.139. This value is then multiplied by the dilution ratio, which in this example is 2, and provided a final colour value of 718 mg/L.

DOC and TOC

Originally, the DOC and TOC values were provided by the instrument, however, due to instrument malfunction midway through the research, measurement of plotted peak height was then used. Peak height measurement of standards was used to determine the DOC and TOC values. The following is a worked example of this TOC value determination. Table A.3 lists the values used.

Table A.3 Worked example values

Sample	Height of peak (mm)				Value	Average
Blank	5	8	5	5	6	
Std. 50	16	15	17	17	16	
Std. 500	158	138	130	127	138	
t=0	67	66	67		67	
t=0	63	60	63		62	64

Using Microsoft® Excel® 2004 a linear equation was determined using the standard values. For the values of obtain in Table A.3 the Equation A.3 was found.

$$y = 3.0119x + 6.3303 \quad \text{(A.3)}$$

Where x represents the peak height value and y is the DOC or TOC value (mg/L). This equation has a R^2=0.9905 and provided a y value of 223 mg/L for x of 64.

TSS

TSS values were determined by the following Equation A4.

$$TSS = \frac{\left[\left(W_{sample} - W_{filter}\right) - W_{blank}\right]}{V} \quad \text{(A4)}$$

Where W_{sample} represents the dried weight of the filter containing the solids of the sample and the filter dish, W_{filter} stands for the blank filter and filter dish prior to filtering and drying, W_{blank} indicates a filter that passed distilled water at the same volume as the sample and then dried, and V represents the volume of sample.

In the practical application study for Blank 1 W_{sample} was 1.1218 g, W_{filter} was 1.1215 g, W_{blank} was -0.0003 g and V is 0.015 L. This provided a TSS value of 40 mg/L.

BOD_5

BOD_5 values were determined using Equation A5.

$$BOD_5 = \left[\frac{(DO_{initial} - DO_{final})}{V_{sample}/V_{bottle}}\right] - BOD_{dilution} \quad \text{(A5)}$$

Where DO_{initial} stands for initial dissolved oxygen content, DO_{final} indicates final dissolved oxygen content, $BOD_{dilution}$ represents the BOD_5 of the dilution water, V_{sample} describes the volume of sample added to the bottle, and V_{bottle} is the total volume of the BOD bottle.

In the practical application study for the 4.0 g sample DO_{initial} was 9.81 mg/L, DO_{final} was 3.72 mg/L, $BOD_{dilution}$ was 1.91 mg/L, V_{sample} was 0.5 mL and V_{bottle} was 60 mL. This provided a BOD_5 value of 729 mg/L.

APPENDIX 2. EFFLUENT CHARACTERIZATION DATA

Table A.5 Electrical conductivity measurement on raw Western Pulp effluent

Sample	micro Siemens
1	2100

Table A.6 Chloride measurement on raw Western Pulp effluent

Sample	Chloride (mg/L)
1	156
2	155

Table A.7 Electrical conductivity measurement on raw Howe Sound effluent

Sample	micro Siemens
1	3000

Table A.8 Chloride measurement on raw Howe Sound effluent

Sample	Chloride (mg/L)
1	145
2	146

APPENDIX 3. MOLECULAR WEIGHT DISTRIBUTION DATA

Table A.9 Colour measurement for the molecular weight distribution on Western Pulp effluent

Sample	Absorbance
Blank	0
Std. 50	0.024
Std. 100	0.039
Std. 300	0.082
Std. 500	0.143
500 Daltons	0.027
1,000 Daltons	0.033
3,000 Daltons	0.035
10,000 Daltons	0.038
100,000 Daltons	0.093
Total	0.114

Note: absorbance measured on Unican UV 300

Table A.10 TOC measurement for the molecular weight distribution on Western Pulp effluent

Sample	Instrument Value	Average
Blank	0	
500 Daltons	95	
500 Daltons	95	
500 Daltons	97	96
1000 Daltons	96	
1000 Daltons	99	
1000 Daltons	97	97
3000 Daltons	109	
3000 Daltons	97	
3000 Daltons	98	101
10000 Daltons	98	
10000 Daltons	97	
10000 Daltons	94	96
100000 Daltons	160	
100000 Daltons	173	
100000 Daltons	165	166
Total	193	
Total	179	186

Table A.11 Colour measurement for the molecular weight distribution on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.018	0.019	0.019	
Std. 100	0.042	0.042	0.042	
Std. 300	0.105	0.105	0.105	
Std. 500	0.177	0.177	0.177	
500 Daltons	0.005	0.004	0.005	0.005
1000 Daltons	0.006	0.007	0.007	
1000 Daltons	0.006	0.007	0.007	0.007
3000 Daltons	0.008	0.008	0.008	
3000 Daltons	0.01	0.008	0.009	0.009
10000 Daltons	0.019	0.02	0.020	
10000 Daltons	0.018	0.016	0.017	0.018
100000 Daltons	0.062	0.064	0.063	
100000 Daltons	0.064	0.065	0.065	0.064
Total	0.11	0.111	0.111	
Total	0.129	0.131	0.130	0.120

Table A.12 TOC measurement for the molecular weight distribution on Howe Sound effluent

Sample	Instrument Value (mg/L)			Average (mg/L)
500 Daltons	32	32	32	
500 Daltons	39	40	39	36
1000 Daltons	33	35	34	
1000 Daltons	32	32	34	33
3000 Daltons	33	33	39	
3000 Daltons	33	33	32	34
10000 Daltons	32	32	32	
10000 Daltons	30	29	32	31
100000 Daltons	50	51	47	
100000 Daltons	49	51	49	50
Total	58	60	60	
Total	55	56	57	58

APPENDIX 4. PRETREATMENT MINI-STUDY DATA

Table A.13 Colour measurement at 465 nm for Pretreatment mini-study on Western Pulp effluent

Sample	Absorbance			Value	Average
Blank	0			0	
Std. 50	0.023	0.025		0.024	
Std. 100	0.051	0.053		0.052	
Std. 300	0.116	0.115		0.116	
Std. 500	0.190	0.192		0.191	
HCl	0.142	0.140	0.141	0.141	
HCl	0.141	0.142	0.140	0.141	
HCl	0.135	0.137		0.136	
HCl	0.137	0.139		0.138	0.139
Filter Blank	0.152	0.149	0.152	0.151	
Filter Blank	0.152	0.152		0.152	0.152
Autoclaved	0.196	0.198	0.198	0.197	
Autoclaved	0.196	0.197		0.197	0.197*
Autoclaved	0.119	0.120	0.118	0.119	
Autoclaved	0.125	0.130	0.132	0.129	0.124
NaOH	0.121	0.118		0.120	
NaOH	0.114	0.118	0.118	0.117	
NaOH	0.107	0.106		0.107	
NaOH	0.106	0.112		0.109	0.113
H_2SO_4	0.129	0.128		0.129	
H_2SO_4	0.124	0.123		0.124	
H_2SO_4	0.130	0.131		0.131	
H_2SO_4	0.128	0.128		0.128	0.128
NaCl	0.167	0.163		0.165	
NaCl	0.163	0.164		0.164	
NaCl	0.138	0.143	0.143	0.141	
NaCl	0.138	0.138		0.138	0.152
$NaHCO_3$	0.108	0.109		0.109	
$NaHCO_3$	0.109	0.115	0.109	0.111	
$NaHCO_3$	0.152	0.149	0.152	0.151	
$NaHCO_3$	0.151	0.15	0.148	0.150	0.130
$CaCl_2$	0.118	0.119		0.119	
$CaCl_2$	0.121	0.119	0.118	0.119	
$CaCl_2$	0.121	0.124	0.120	0.122	
$CaCl_2$	0.12	0.123	0.118	0.120	0.120
Na_2CO_3	0.129	0.130		0.130	
Na_2CO_3	0.132	0.133		0.133	
Na_2CO_3	0.132	0.128	0.129	0.130	
Na_2CO_3	0.119	0.119		0.119	0.128

* Assumed filter breakthrough

Table A.14 Colour measurement at 400 nm for Pretreatment mini-study on Western Pulp effluent

Sample	Absorbance			Value	Average
Blank	0	0		0	
HCl	0.178	0.174	0.174	0.175	
HCl	0.187	0.186	0.185	0.186	
HCl	0.181	0.178	0.183	0.181	
HCl	0.178	0.178	0.177	0.178	0.180
Filter Blank	0.263	0.253	0.258	0.258	
Filter Blank	0.256	0.258	0.257	0.257	0.258
Autoclaved	0.206	0.208	0.206	0.207	
Autoclaved	0.219	0.216	0.215	0.217	0.212*
Autoclaved	0.17	0.175	0.173	0.173	
Autoclaved	0.182	0.183	0.185	0.183	0.178
NaOH	0.204	0.205	0.200	0.203	
NaOH	0.204	0.197	0.200	0.200	
NaOH	0.183	0.186	0.184	0.184	
NaOH	0.181	0.184	0.183	0.183	0.193
H_2SO_4	0.209	0.209	0.210	0.209	
H_2SO_4	0.211	0.214	0.214	0.213	
H_2SO_4	0.185	0.186	0.184	0.185	
H_2SO_4	0.186	0.183	0.184	0.185	0.198
NaCl	0.200	0.200	0.200	0.200	
NaCl	0.204	0.201	0.204	0.203	
NaCl	0.196	0.195	0.190	0.194	
NaCl	0.199	0.202	0.197	0.199	0.199
$NaHCO_3$	0.184	0.186	0.181	0.184	
$NaHCO_3$	0.202	0.209	0.193	0.201	
$NaHCO_3$	0.193	0.187	0.189	0.190	
$NaHCO_3$	0.178	0.178	0.176	0.177	0.188
$CaCl_2$	0.202	0.204	0.195	0.200	
$CaCl_2$	0.201	0.205	0.198	0.201	
$CaCl_2$	0.192	0.198	0.194	0.195	
$CaCl_2$	0.202	0.199	0.203	0.201	0.199
Na_2CO_3	0.226	0.227	0.230	0.228	
Na_2CO_3	0.225	0.225	0.226	0.225	
Na_2CO_3	0.220	0.219	0.226	0.222	
Na_2CO_3	0.208	0.206	0.209	0.208	0.221

* Assumed filter breakthrough

Table A.15 COD measurement at 600 nm for Pretreatment mini-study on Western Pulp effluent

Sample	Absorbance			Value	Average
Blank	0			0	
Std. 100	0. 036			0. 036	
Std. 300	0.114			0.114	
Std. 500	0.197			0.197	
Std. 800	0.317			0.317	
HCl	0.146	0.145	0.145	0.145	
HCl	0.141	0.141		0.141	
HCl	0.137	0.137		0.137	
HCl	0.134	0.134		0.134	0.139
Filter Blank	0.125	0.126		0.126	
Filter Blank	0.125	0.125		0.125	0.125
Autoclaved	0.175	0.175		0.175	
Autoclaved	0.178	0.178		0.178	0.177*
Autoclaved	0.153	0.152		0.153	
Autoclaved	0.154	0.154		0.154	0.153
NaOH	0.129	0.129		0.129	
NaOH	0.125	0.125		0.125	
NaOH	0.12	0.119		0.120	
NaOH	0.123	0.123		0.123	0.124
H_2SO_4	0.121	0.125	0.121	0.122	
H_2SO_4	0.122	0.121		0.122	
H_2SO_4	0.145	0.145		0.145	
H_2SO_4	0.148	0.148		0.148	0.134
NaCl	0.152	0.151		0.152	
NaCl	0.151	0.15		0.151	
NaCl	0.144	0.143		0.144	
NaCl	0.137	0.136		0.137	0.146
$NaHCO_3$	0.122	0.122		0.122	
$NaHCO_3$	0.121	0.121		0.121	
$NaHCO_3$	0.143	0.143		0.143	
$NaHCO_3$	0.146	0.142		0.144	0.133
$CaCl_2$	0.124	0.123		0.124	
$CaCl_2$	0.123	0.123		0.123	
$CaCl_2$	0.121	0.124		0.123	
$CaCl_2$	0.123	0.124		0.124	0.123
Na_2CO_3	0.138	0.138		0.138	
Na_2CO_3	0.136	0.139		0.138	
Na_2CO_3	0.131	0.132		0.132	
Na_2CO_3	0.127	0.127		0.127	0.134

* Assumed filter breakthrough

Table A.16 pH measurement for Pretreatment mini-study results on Western Pulp effluent

Pre-treatment	pH
Blanks	8.69
Autoclaved	8.05
Autoclaved	8.31
HCl	8.00
HCl	7.90
NaOH	8.26
NaOH	8.62
H_2SO_4	8.32
H_2SO_4	8.38
NaCl	8.06
NaCl	8.18
$NaHCO_3$	8.38
$NaHCO_3$	8.14
$CaCl_2$	8.35
$CaCl_2$	8.36
Na_2CO_3	8.14
Na_2CO_3	8.30

APPENDIX 5. PH MINI-STUDY DATA

Table A.17 Colour measurement for pH mini-study on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.016	0.016	
Std. 100	0.042	0.043	0.043	
Std. 300	0.107	0.107	0.107	
Std. 500	0.184	0.184	0.184	
pH 2	0.019	0.015	0.017	
pH 2	0.018	0.018	0.018	
pH 2	0.016	0.017	0.017	
pH 2	0.014	0.016	0.015	0.017
pH 4	0.040	0.037	0.039	
pH 4	0.040	0.040	0.040	
pH 4	0.046	0.048	0.047	
pH 4	0.040	0.040	0.040	0.041
pH 6	0.090	0.089	0.090	
pH 6	0.086	0.087	0.087	
pH 6	0.097	0.096	0.097	
pH 6	0.095	0.094	0.095	0.092
pH 8	0.061	0.061	0.061	
pH 8	0.066	0.066	0.066	
pH 8	0.074	0.072	0.073	
pH 8	0.073	0.075	0.074	0.069
pH 10	0.081	0.081	0.081	
pH 10	0.080	0.080	0.080	
pH 10	0.081	0.082	0.082	
pH 10	0.082	0.085	0.084	0.082
pH 12	0.094	0.092	0.093	
pH 12	0.095	0.093	0.094	
pH 12	0.090	0.090	0.090	
pH 12	0.093	0.093	0.093	0.093
Original pH	0.083	0.084	0.084	
Original pH	0.085	0.081	0.083	0.083
Filter blank	0.127	0.125	0.126	
Filter blank	0.129	0.127	0.128	0.127

Table A.18 COD measurement for pH mini-study results on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0	0	0	
Std. 100	0.036	0.036	0.036	
Std. 300	0.114	0.115	0.115	
Std. 500	0.197	0.195	0.196	
Std. 800	0.318	0.317	0.318	
pH 2	0.156	0.156	0.156	
pH 2	0.156	0.160	0.158	
pH 2	0.122	0.121	0.122	
pH 2	0.122	0.122	0.122	0.139
pH 4	0.150	0.149	0.150	
pH 4	0.152	0.149	0.151	
pH 4	0.122	0.122	0.122	
pH 4	0.123	0.123	0.123	0.136
pH 6	0.110	0.109	0.110	
pH 6	0.102	0.103	0.103	
pH 6	0.107	0.103	0.105	
pH 6	0.097	0.098	0.098	0.104
pH 8	0.124	0.123	0.124	
pH 8	0.121	0.123	0.122	
pH 8	0.125	0.126	0.126	
pH 8	0.127	0.129	0.128	0.125
pH 10	0.137	0.137	0.137	
pH 10	0.136	0.137	0.137	
pH 10	0.149	0.148	0.149	
pH 10	0.151	0.150	0.151	0.143
pH 12	0.284	0.283	0.284	
pH 12	0.265	0.266	0.266	
pH 12	0.271	0.272	0.272	
pH 12	0.277	0.277	0.277	0.274
Original pH	0.122	0.123	0.123	
Original pH	0.120	0.120	0.120	0.121
Filter blank	0.120	0.118	0.119	
Filter blank	0.121	0.123	0.122	0.121

APPENDIX 6. BIOMASS WASHING MINI-STUDY

Table A.19 Colour measurement for original biomass wash method on de-ionized water

Sample	Absorbance			Value	Average
Blank	0			0	
Std. 50	0.018	0.017		0.018	
Std. 100	0.047	0.047		0.047	
Std. 300	0.110	0.110		0.110	
Std. 500	0.185	0.184		0.185	
t=0.25 h	0.019	0.019		0.019	
t=0.25 h	0.026	0.027		0.027	0.023
t=24 h	0.015	0.014		0.015	
t=24 h	0.026	0.029	0.031	0.029	0.022
t=48 h	0.005	0.006	0.006	0.006	
t=48 h	0.002	0.002		0.002	0.004
Filter blank t=48 h	0.001	0.003		0.002	
Filter blank t=48 h	0.001	0.001		0.001	0.002

Table A.20 COD measurement for original biomass wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.036	0.036	0.036	
Std. 300	0.115	0.115	0.115	
Std. 500	0.196	0.196	0.196	
Std. 800	0.317	0.317	0.317	
t=0.25 h	0.041	0.041	0.041	
t=0.25 h	0.045	0.045	0.045	0.043
t=24 h	0.062	0.062	0.062	
t=24 h	0.063	0.062	0.063	0.062
t=48 h	0.050	0.050	0.050	
t=48 h	0.048	0.048	0.048	0.049
Filter blank t=48 h	0.003	0.002	0.003	
Filter blank t=48 h	0.003	0.001	0.002	0.002

Table A.21 Test run 1 colour measurement for "after autoclave" biomass wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.017	0.017	0.017	
Std. 100	0.041	0.040	0.041	
Std. 300	0.106	0.106	0.106	
Std. 500	0.178	0.178	0.178	
t=0.25 h	0.005	0.003	0.004	
t=0.25 h	0.002	0.003	0.003	0.003
t=1 h	0.003	0.001	0.002	
t=1 h	0.004	0.001	0.003	0.002
t=4 h	0.001	0.003	0.002	
t=4 h	0.006	0.004	0.005	0.004
t=24 h	0.001	0.002	0.002	
t=24 h	0.003	0.002	0.003	0.002
t=48 h	0.001	0.003	0.002	
t=48 h	0.005	0.003	0.004	0.003
Filter blank t=48 h	0.001	0.000	0.001	
Filter blank t=48 h	0.002	0.003	0.003	0.002

Table A.22 Test run 1 COD measurement for "after autoclave" wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.035	0.035	0.035	
Std. 300	0.114	0.114	0.114	
Std. 500	0.196	0.196	0.196	
Std. 800	0.316	0.316	0.316	
t=0.25 h	0.038	0.038	0.038	
t=0.25 h	0.037	0.036	0.037	0.037
t=1 h	0.064	0.064	0.064	
t=1 h	0.065	0.065	0.065	0.065
t=4 h	0.029	0.028	0.029	
t=4 h	0.030	0.030	0.030	0.029
t=24 h	0.025	0.025	0.025	
t=24 h	0.026	0.026	0.026	0.026
t=48 h	0.032	0.030	0.031	
t=48 h	0.024	0.023	0.024	0.027
Filter blank t=48 h	0.002	0.000	0.001	
Filter blank t=48 h	-0.001	-0.001	-0.001	0

Table A.23 Test run 2 colour measurement for "after autoclave" biomass wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.013	0.012	0.013	
Std. 100	0.037	0.036	0.037	
Std. 300	0.103	0.101	0.102	
Std. 500	0.178	0.177	0.178	
t=0.25 h	0.000	0.000	0.000	
t=0.25 h	0.001	0.000	0.001	0.000
t=1 h	0.004	0.003	0.004	
t=1 h	0.001	0.000	0.001	0.002
t=4 h	0.001	0.003	0.002	
t=4 h	0.002	0.002	0.002	0.002
t=24 h	0.003	0.004	0.004	
t=24 h	0.005	0.005	0.005	0.004
t=48 h	0.002	0.003	0.003	
t=48 h	0.003	0.002	0.003	0.003
Filter blank t=48 h	0.002	0.004	0.003	
Filter blank t=48 h	0.005	0.005	0.005	0.004

Table A.24 Test run 2 COD measurement for "after autoclave" wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.03	0.03	0.030	
Std. 300	0.112	0.112	0.112	
Std. 500	0.201	0.201	0.201	
Std. 800	0.310	0.310	0.310	
t=0.25 h	0.023	0.022	0.023	
t=0.25 h	0.022	0.022	0.022	0.022
t=1 h	0.033	0.030	0.032	
t=1 h	0.021	0.019	0.020	0.026
t=4 h	0.025	0.024	0.025	
t=4 h	0.021	0.021	0.021	0.023
t=24 h	0.033	0.032	0.033	
t=24 h	0.033	0.030	0.032	0.032
t=48 h	0.032	0.032	0.032	
t=48 h	0.032	0.032	0.032	0.032
Filter blank t=48 h	-0.002	-0.003	-0.003	
Filter blank t=48 h	0.000	0.001	0.001	-0.001

Table A.25 Test run 1 colour measurement for "double wash" biomass wash method on de-ionized water

Sample	Absorbance			Value	Average
Blank	0			0	
Std. 50	0.016	0.017		0.017	
Std. 100	0.042	0.043		0.043	
Std. 300	0.108	0.108		0.108	
Std. 500	0.185	0.185		0.185	
t=0.25 h	0.001	0.001		0.001	
t=0.25 h	0.003	0.005		0.004	0.003
t=24 h	0.000	0.001		0.001	
t=24 h	0.000	0.000		0.000	0.000
t=48 h	0.001	0.001		0.001	
t=48 h	0.005	0.001	0.003	0.003	0.002
Filter blank t=48 h	0.001	0.002		0.002	
Filter blank t=48 h	-0.001	-0.001		-0.001	0.000

Table A.26 Test run 1 COD measurement for "double wash" biomass wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.035	0.034	0.035	
Std. 300	0.114	0.114	0.114	
Std. 500	0.196	0.196	0.196	
Std. 800	0.315	0.315	0.315	
t=0.25 h	0.008	0.008	0.008	
t=0.25 h	0.008	0.007	0.008	0.008
t=24 h	0.013	0.013	0.013	
t=24 h	0.012	0.012	0.012	0.013
t=48 h	0.005	0.004	0.005	
t=48 h	0.008	0.008	0.008	0.006
Filter blank t=48 h	-0.002	-0.002	-0.002	
Filter blank t=48 h	-0.001	0.001	0.000	-0.001

Table A.27 Test run 2 colour measurement for "double wash" biomass wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.019	0.017	0.018	
Std. 100	0.040	0.041	0.041	
Std. 300	0.109	0.105	0.107	
Std. 500	0.178	0.177	0.178	
t=0.25 h	0.004	0.002	0.003	
t=0.25 h	0.006	0.001	0.004	0.003
t=1 h	0.004	0.002	0.003	
t=1 h	0.004	0.005	0.005	0.004
t=24 h	0.004	0.004	0.004	
t=24 h	0.004	0.005	0.005	0.004
t=48 h	0.004	0.006	0.005	
t=48 h	0.007	0.004	0.006	0.005
Filter blank t=48 h	0.004	0.002	0.003	
Filter blank t=48 h	0.006	0.001	0.004	0.003

Table A.28 Test run 2 COD measurement for "double wash" biomass wash method on de-ionized water

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.033	0.033	0.033	
Std. 300	0.115	0.115	0.115	
Std. 500	0.204	0.205	0.205	
Std. 800	0.315	0.314	0.315	
t=0.25 h	0.008	0.008	0.008	
t=0.25 h	0.009	0.010	0.010	0.009
t=1 h	0.010	0.010	0.010	
t=1 h	0.011	0.009	0.010	0.010
t=24 h	0.008	0.008	0.008	
t=24 h	0.008	0.008	0.008	0.008
t=48 h	0.015	0.017	0.016	
t=48 h	0.017	0.017	0.017	0.017
Filter blank t=48 h	0.002	0.001	0.002	
Filter blank t=48 h	0.005	0.006	0.006	0.004

APPENDIX 7. MIXING MINI-STUDY

Table A.29 Test run 1 colour measurement mixing study 300 mL flask at 125 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.019	0.016	0.018	
Std. 100	0.039	0.041	0.040	
Std. 300	0.102	0.103	0.103	
Std. 500	0.176	0.176	0.176	
d=0, t=0	0.124	0.126	0.125	
d=0, t=0	0.122	0.124	0.123	0.124
d=0.8 g, t=0.25 h	0.093	0.092	0.093	
d=0.8 g, t=0.25 h	0.088	0.090	0.089	0.091
d=0.5 g, t=0.25 h	0.099	0.103	0.101	
d=0.5 g, t=0.25 h	0.098	0.101	0.100	0.100
d=0.2 g, t=0.25 h	0.104	0.103	0.104	
d=0.2 g, t=0.25 h	0.102	0.102	0.102	0.103
d=0.8 g, t=1 h	0.081	0.081	0.081	
d=0.8 g, t=1 h	0.079	0.078	0.079	0.080
d=0.5 g, t=1 h	0.091	0.088	0.090	
d=0.5 g, t=1 h	0.092	0.094	0.093	0.091
d=0.2 g, t=1 h	0.098	0.096	0.097	
d=0.2 g, t=1 h	0.091	0.092	0.092	0.094
d=0.8 g, t=4 h	0.083	0.083	0.083	
d=0.8 g, t=4 h	0.077	0.079	0.078	0.081
d=0.5 g, t=4 h	0.088	0.088	0.088	
d=0.5 g, t=4 h	0.085	0.084	0.085	0.086
d=0.2 g, t=4 h	0.097	0.096	0.097	
d=0.2 g, t=4 h	0.097	0.098	0.098	0.097
d=0.8 g, t=24 h	0.077	0.075	0.076	
d=0.8 g, t=24 h	0.077	0.076	0.077	0.076
d=0.5 g, t=24 h	0.082	0.082	0.082	
d=0.5 g, t=24 h	0.083	0.083	0.083	0.083
d=0.2 g, t=24 h	0.089	0.089	0.089	
d=0.2 g, t=24 h	0.086	0.088	0.087	0.088
d=0, t=24 h	0.119	0.120	0.120	
d=0, t=24 h	0.119	0.116	0.118	0.119

Table A.30 Test run 1 COD measurement mixing study 300 mL flask at 125 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.022	0.023	0.023	
Std. 300	0.116	0.117	0.117	
Std. 500	0.195	0.196	0.196	
Std. 800	0.313	0.313	0.313	
d=0, t=0	0.114	0.114	0.114	
d=0, t=0	0.112	0.112	0.112	0.113
d=0.8 g, t=0.25 h	0.167	0.166	0.167	
d=0.8 g, t=0.25 h	0.174	0.175	0.175	0.171
d=0.5 g, t=0.25 h	0.158	0.158	0.158	
d=0.5 g, t=0.25 h	0.144	0.143	0.144	0.151
d=0.2 g, t=0.25 h	0.123	0.123	0.123	
d=0.2 g, t=0.25 h	0.126	0.126	0.126	0.125
d=0.8 g, t=1 h	0.177	0.177	0.177	
d=0.8 g, t=1 h	0.175	0.176	0.176	0.176
d=0.5 g, t=1 h	0.160	0.159	0.160	
d=0.5 g, t=1 h	0.159	0.159	0.159	0.159
d=0.2 g, t=1 h	0.124	0.124	0.124	
d=0.2 g, t=1 h	0.126	0.126	0.126	0.125
d=0.8 g, t=4 h	0.184	0.188	0.186	
d=0.8 g, t=4 h	0.226	0.226	0.226	0.206
d=0.5 g, t=4 h	0.164	0.166	0.165	
d=0.5 g, t=4 h	0.164	0.164	0.164	0.165
d=0.2 g, t=4 h	0.130	0.129	0.130	
d=0.2 g, t=4 h	0.129	0.131	0.130	0.130
d=0.8 g, t=24 h	0.231	0.231	0.231	
d=0.8 g, t=24 h	0.232	0.236	0.234	0.233
d=0.5 g, t=24 h	0.191	0.191	0.191	
d=0.5 g, t=24 h	0.192	0.191	0.192	0.191
d=0.2 g, t=24 h	0.137	0.139	0.138	
d=0.2 g, t=24 h	0.139	0.140	0.140	0.139
d=0, t=24 h	0.125	0.124	0.125	
d=0, t=24 h	0.124	0.124	0.124	0.124

Table A.31 Test run 1 DOC measurement mixing study 300 mL flask at 125 rpm

Sample	DOC (mg/L)	Average (mg/L)
d=0, t=0	283	
d=0, t=0	239	261
d=0.8 g, t=0.25 h	373	
d=0.8 g, t=0.25 h	393	383
d=0.5 g, t=0.25 h	322	
d=0.5 g, t=0.25 h	311	317
d=0.2 g, t=0.25 h	294	
d=0.2 g, t=0.25 h	269	282
d=0.8 g, t=1 h	379	
d=0.8 g, t=1 h	389	384
d=0.5 g, t=1 h	328	
d=0.5 g, t=1 h	334	331
d=0.2 g, t=1 h	270	
d=0.2 g, t=1 h	287	279
d=0.8 g, t=4 h	403	
d=0.8 g, t=4 h	377	390
d=0.5 g, t=4 h	345	
d=0.5 g, t=4 h	339	342
d=0.2 g, t=4 h	259	
d=0.2 g, t=4 h	227	243
d=0.8 g, t=24 h	414	
d=0.8 g, t=24 h	413	414
d=0.5 g, t=24 h	264	
d=0.5 g, t=24 h	311	288
d=0.2 g, t=24 h	247	
d=0.2 g, t=24 h	243	245
d=0, t=24 h	214	
d=0, t=24 h	165	190

Table A.32 Test run 2 colour measurement mixing study 300 mL flask at 125 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.018	0.017	
Std. 100	0.040	0.041	0.041	
Std. 300	0.102	0.102	0.102	
Std. 500	0.177	0.175	0.176	
d=0.8 g, t=0.25 h	0.090	0.091	0.091	
d=0.8 g, t=0.25 h	0.091	0.091	0.091	0.091
d=0.5 g, t=0.25 h	0.101	0.099	0.100	
d=0.5 g, t=0.25 h	0.101	0.101	0.101	0.101
d=0.2 g, t=0.25 h	0.102	0.104	0.103	
d=0.2 g, t=0.25 h	0.103	0.104	0.104	0.103
d=0.8 g, t=1 h	0.082	0.081	0.082	
d=0.8 g, t=1 h	0.081	0.082	0.082	0.082
d=0.5 g, t=1 h	0.098	0.097	0.098	
d=0.5 g, t=1 h	0.096	0.097	0.097	0.097
d=0.2 g, t=1 h	0.108	0.107	0.108	
d=0.2 g, t=1 h	0.102	0.105	0.104	0.106
d=0.8 g, t=4 h	0.073	0.074	0.074	
d=0.8 g, t=4 h	0.076	0.077	0.077	0.075
d=0.5 g, t=4 h	0.090	0.088	0.089	
d=0.5 g, t=4 h	0.090	0.088	0.089	0.089
d=0.2 g, t=4 h	0.095	0.099	0.097	
d=0.2 g, t=4 h	0.102	0.101	0.102	0.099
d=0.8 g, t=24 h	0.080	0.080	0.080	
d=0.8 g, t=24 h	0.074	0.074	0.074	0.077
d=0.5 g, t=24 h	0.077	0.077	0.077	
d=0.5 g, t=24 h	0.081	0.081	0.081	0.079
d=0.2 g, t=24 h	0.088	0.089	0.089	
d=0.2 g, t=24 h	0.089	0.088	0.089	0.089
d=0, t=24 h	0.124	0.126	0.125	
d=0, t=24 h	0.121	0.121	0.121	0.123

Table A.33 Test run 2 COD measurement mixing study 300 mL flask at 125 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.025	0.025	0.025	
Std. 300	0.115	0.116	0.116	
Std. 500	0.195	0.194	0.195	
Std. 800	0.311	0.312	0.312	
d=0, t=0	0.114	0.114	0.114	
d=0, t=0	0.112	0.112	0.112	0.113
d=0.8 g, t=0.25 h	0.156	0.157	0.157	
d=0.8 g, t=0.25 h	0.164	0.162	0.163	0.160
d=0.5 g, t=0.25 h	0.145	0.014	0.080	
d=0.5 g, t=0.25 h	0.149	0.149	0.149	0.114
d=0.2 g, t=0.25 h	0.124	0.123	0.124	
d=0.2 g, t=0.25 h	0.117	0.116	0.117	0.120
d=0.8 g, t=1 h	0.161	0.163	0.162	
d=0.8 g, t=1 h	0.161	0.162	0.162	0.162
d=0.5 g, t=1 h	0.143	0.143	0.143	
d=0.5 g, t=1 h	0.150	0.149	0.150	0.146
d=0.2 g, t=1 h	0.121	0.122	0.122	
d=0.2 g, t=1 h	0.120	0.119	0.120	0.121
d=0.8 g, t=4 h	0.187	0.181	0.184	
d=0.8 g, t=4 h	0.180	0.182	0.181	0.183
d=0.5 g, t=4 h	0.155	0.156	0.156	
d=0.5 g, t=4 h	0.165	0.163	0.164	0.160
d=0.2 g, t=4 h	0.122	0.118	0.120	
d=0.2 g, t=4 h	0.119	0.120	0.120	0.120
d=0.8 g, t=24 h	0.205	0.207	0.206	
d=0.8 g, t=24 h	0.214	0.213	0.214	0.210
d=0.5 g, t=24 h	0.164	0.163	0.164	
d=0.5 g, t=24 h	0.175	0.175	0.175	0.169
d=0.2 g, t=24 h	0.127	0.128	0.128	
d=0.2 g, t=24 h	0.121	0.123	0.122	0.125
d=0, t=24 h	0.122	0.122	0.122	
d=0, t=24 h	0.123	0.123	0.123	0.123

Table A.34 Test run 2 DOC measurement mixing study 300 mL flask at 125 rpm

Sample	DOC (mg/L)	Average (mg/L)
d=0, t=0	283	
d=0, t=0	239	261
d=0.8 g, t=0.25 h	289	
d=0.8 g, t=0.25 h	275	282
d=0.5 g, t=0.25 h	265	
d=0.5 g, t=0.25 h	264	265
d=0.2 g, t=0.25 h	217	
d=0.2 g, t=0.25 h	218	218
d=0.8 g, t=1 h	291	
d=0.8 g, t=1 h	285	288
d=0.5 g, t=1 h	270	
d=0.5 g, t=1 h	267	269
d=0.2 g, t=1 h	226	
d=0.2 g, t=1 h	207	217
d=0.8 g, t=4 h	282	
d=0.8 g, t=4 h	267	275
d=0.5 g, t=4 h	264	
d=0.5 g, t=4 h	254	259
d=0.2 g, t=4 h	212	
d=0.2 g, t=4 h	207	210
d=0.8 g, t=24 h	293	
d=0.8 g, t=24 h	295	294
d=0.5 g, t=24 h	292	
d=0.5 g, t=24 h	284	288
d=0.2 g, t=24 h	219	
d=0.2 g, t=24 h	211	215
d=0, t=24 h	189	
d=0, t=24 h	206	198

Table A.35 Test run 1 colour measurement mixing study 300 mL flask at 200 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.017	0.018	0.018	
Std. 100	0.042	0.040	0.041	
Std. 300	0.105	0.103	0.104	
Std. 500	0.178	0.178	0.178	
d=0.8 g, t=0.25 h	0.087	0.088	0.088	
d=0.8 g, t=0.25 h	0.084	0.083	0.084	0.086
d=0.5 g, t=0.25 h	0.113	0.116	0.115	
d=0.5 g, t=0.25 h	0.117	0.117	0.117	0.116
d=0.2 g, t=0.25 h	0.113	0.110	0.112	
d=0.2 g, t=0.25 h	0.107	0.111	0.109	0.110
d=0.8 g, t=1 h	0.095	0.096	0.096	
d=0.8 g, t=1 h	0.097	0.094	0.096	0.096
d=0.5 g, t=1 h	0.105	0.104	0.105	
d=0.5 g, t=1 h	0.095	0.097	0.096	0.100
d=0.2 g, t=1 h	0.108	0.105	0.107	
d=0.2 g, t=1 h	0.107	0.109	0.108	0.107
d=0.8 g, t=4 h	0.092	0.095	0.094	
d=0.8 g, t=4 h	0.088	0.089	0.089	0.091
d=0.5 g, t=4 h	0.082	0.083	0.083	
d=0.5 g, t=4 h	0.088	0.087	0.088	0.085
d=0.2 g, t=4 h	0.104	0.104	0.104	
d=0.2 g, t=4 h	0.104	0.103	0.104	0.104
d=0.8 g, t=24 h	0.070	0.071	0.071	
d=0.8 g, t=24 h	0.069	0.069	0.069	0.070
d=0.5 g, t=24 h	0.075	0.073	0.074	
d=0.5 g, t=24 h	0.075	0.071	0.073	0.074
d=0.2 g, t=24 h	0.084	0.085	0.085	
d=0.2 g, t=24 h	0.084	0.085	0.085	0.085
d=0, t=24 h	0.121	0.120	0.121	
d=0, t=24 h	0.124	0.121	0.123	0.122

Table A.36 Test run 1 COD measurement mixing study 300 mL flask at 200 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.024	0.023	0.024	
Std. 300	0.114	0.114	0.114	
Std. 500	0.193	0.193	0.193	
Std. 800	0.308	0.309	0.309	
d=0, t=0	0.110	0.110	0.110	
d=0, t=0	0.110	0.111	0.111	0.110
d=0.8 g, t=0.25 h	0.174	0.172	0.173	
d=0.8 g, t=0.25 h	0.168	0.168	0.168	0.171
d=0.5 g, t=0.25 h	0.152	0.150	0.151	
d=0.5 g, t=0.25 h	0.148	0.149	0.149	0.150
d=0.2 g, t=0.25 h	0.123	0.125	0.124	
d=0.2 g, t=0.25 h	0.119	0.118	0.119	0.121
d=0.8 g, t=1 h	0.164	0.165	0.165	
d=0.8 g, t=1 h	0.171	0.170	0.171	0.168
d=0.5 g, t=1 h	0.141	0.142	0.142	
d=0.5 g, t=1 h	0.137	0.137	0.137	0.139
d=0.2 g, t=1 h	0.127	0.125	0.126	
d=0.2 g, t=1 h	0.123	0.126	0.125	0.125
d=0.8 g, t=4 h	0.144	0.144	0.144	
d=0.8 g, t=4 h	0.146	0.146	0.146	0.145
d=0.5 g, t=4 h	0.137	0.136	0.137	
d=0.5 g, t=4 h	0.124	0.128	0.126	0.131
d=0.2 g, t=4 h	0.114	0.112	0.113	
d=0.2 g, t=4 h	0.113	0.112	0.113	0.113
d=0.8 g, t=24 h	0.212	0.215	0.214	
d=0.8 g, t=24 h	0.205	0.206	0.206	0.210
d=0.5 g, t=24 h	0.162	0.161	0.162	
d=0.5 g, t=24 h	0.158	0.157	0.158	0.160
d=0.2 g, t=24 h	0.171	0.171	0.171	
d=0.2 g, t=24 h	0.134	0.134	0.134	0.153
d=0, t=24 h	0.128	0.127	0.128	
d=0, t=24 h	0.120	0.120	0.120	0.124

Table A.37 Test run 1 DOC measurement mixing study 300 mL flask at 200 rpm

Sample	DOC (mg/L)		Average (mg/L)
d=0.8 g, t=0.25 h	293	278	
d=0.8 g, t=0.25 h	265	250	279
d=0.5 g, t=0.25 h	272	257	
d=0.5 g, t=0.25 h	257	242	265
d=0.2 g, t=0.25 h	217	202	
d=0.2 g, t=0.25 h	210	195	214
d=0.8 g, t=1 h	236	221	
d=0.8 g, t=1 h	265	250	251
d=0.5 g, t=1 h	270	255	
d=0.5 g, t=1 h	271	256	271
d=0.2 g, t=1 h	232	217	
d=0.2 g, t=1 h	223	208	228
d=0.8 g, t=4 h	251	236	
d=0.8 g, t=4 h	251	236	251
d=0.5 g, t=4 h	268	253	
d=0.5 g, t=4 h	251	236	260
d=0.2 g, t=4 h	222	207	
d=0.2 g, t=4 h	225	210	224
d=0.8 g, t=24 h	230	215	
d=0.8 g, t=24 h	251	236	241
d=0.5 g, t=24 h	230	215	
d=0.5 g, t=24 h	251	236	241
d=0.2 g, t=24 h	204	189	
d=0.2 g, t=24 h	211	196	208
d=0, t=24 h	202	187	
d=0, t=24 h	179	164	191

Table A.38 Test run 2 colour measurement mixing study 300 mL flask at 200 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.015	0.015	
Std. 100	0.040	0.040	0.040	
Std. 300	0.101	0.102	0.102	
Std. 500	0.174	0.175	0.175	
d=0, t=0	0.121	0.119	0.120	
d=0, t=0	0.121	0.119	0.120	0.120
d=0.8 g, t=0.25 h	0.091	0.093	0.092	
d=0.8 g, t=0.25 h	0.088	0.087	0.088	0.090
d=0.5 g, t=0.25 h	0.105	0.104	0.105	
d=0.5 g, t=0.25 h	0.104	0.105	0.105	0.105
d=0.2 g, t=0.25 h	0.107	0.106	0.107	
d=0.2 g, t=0.25 h	0.108	0.106	0.107	0.107
d=0.8 g, t=1 h	0.086	0.084	0.085	
d=0.8 g, t=1 h	0.082	0.084	0.083	0.084
d=0.5 g, t=1 h	0.092	0.090	0.091	
d=0.5 g, t=1 h	0.094	0.094	0.094	0.093
d=0.2 g, t=1 h	0.112	0.112	0.112	
d=0.2 g, t=1 h	0.106	0.104	0.105	0.109
d=0.8 g, t=4 h	0.083	0.081	0.082	
d=0.8 g, t=4 h	0.081	0.081	0.081	0.082
d=0.5 g, t=4 h	0.100	0.101	0.101	
d=0.5 g, t=4 h	0.103	0.103	0.103	0.102
d=0.2 g, t=4 h	0.089	0.089	0.089	
d=0.2 g, t=4 h	0.098	0.095	0.097	0.093
d=0.8 g, t=24 h	0.062	0.062	0.062	
d=0.8 g, t=24 h	0.064	0.060	0.062	0.062
d=0.5 g, t=24 h	0.076	0.078	0.077	
d=0.5 g, t=24 h	0.076	0.076	0.076	0.077
d=0.2 g, t=24 h	0.085	0.084	0.085	
d=0.2 g, t=24 h	0.086	0.086	0.086	0.085
d=0, t=24 h	0.126	0.123	0.125	
d=0, t=24 h	0.120	0.120	0.120	0.122

Table A.39 Test run 2 COD measurement mixing study 300 mL flask at 200 rpm

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.024	0.023	0.024	
Std. 300	0.114	0.114	0.114	
Std. 500	0.193	0.193	0.193	
Std. 800	0.308	0.309	0.309	
d=0, t=0	0.110	0.110	0.110	
d=0, t=0	0.110	0.111	0.111	0.110
d=0.8 g, t=0.25 h	0.174	0.172	0.173	
d=0.8 g, t=0.25 h	0.168	0.168	0.168	0.171
d=0.5 g, t=0.25 h	0.152	0.150	0.151	
d=0.5 g, t=0.25 h	0.148	0.149	0.149	0.150
d=0.2 g, t=0.25 h	0.123	0.125	0.124	
d=0.2 g, t=0.25 h	0.119	0.118	0.119	0.121
d=0.8 g, t=1 h	0.164	0.165	0.165	
d=0.8 g, t=1 h	0.171	0.170	0.171	0.168
d=0.5 g, t=1 h	0.141	0.142	0.142	
d=0.5 g, t=1 h	0.137	0.137	0.137	0.139
d=0.2 g, t=1 h	0.127	0.125	0.126	
d=0.2 g, t=1 h	0.123	0.126	0.125	0.125
d=0.8 g, t=4 h	0.144	0.144	0.144	
d=0.8 g, t=4 h	0.146	0.146	0.146	0.145
d=0.5 g, t=4 h	0.137	0.136	0.137	
d=0.5 g, t=4 h	0.124	0.128	0.126	0.131
d=0.2 g, t=4 h	0.114	0.112	0.113	
d=0.2 g, t=4 h	0.113	0.112	0.113	0.113
d=0.8 g, t=24 h	0.212	0.215	0.214	
d=0.8 g, t=24 h	0.205	0.206	0.206	0.210
d=0.5 g, t=24 h	0.162	0.161	0.162	
d=0.5 g, t=24 h	0.158	0.157	0.158	0.160
d=0.2 g, t=24 h	0.171	0.171	0.171	
d=0.2 g, t=24 h	0.134	0.134	0.134	0.153
d=0, t=24 h	0.128	0.127	0.128	
d=0, t=24 h	0.120	0.120	0.120	0.124

Table A.40 Test run 2 DOC measurement mixing study 300 mL flask at 200 rpm

Sample	DOC (mg/L)		Average
d=0, t=0	200	186	
d=0, t=0	216	202	208
d=0.8 g, t=0.25 h	275	261	
d=0.8 g, t=0.25 h	215	201	245
d=0.5 g, t=0.25 h	236	222	
d=0.5 g, t=0.25 h	222	208	229
d=0.2 g, t=0.25 h	231	217	
d=0.2 g, t=0.25 h	232	218	232
d=0.8 g, t=1 h	278	264	
d=0.8 g, t=1 h	247	233	263
d=0.5 g, t=1 h	234	220	
d=0.5 g, t=1 h	241	227	238
d=0.2 g, t=1 h	207	193	
d=0.2 g, t=1 h	197	183	202
d=0.8 g, t=4 h	234	229	
d=0.8 g, t=4 h	218	204	226
d=0.5 g, t=4 h	242	237	
d=0.5 g, t=4 h	217	203	230
d=0.2 g, t=4 h	215	201	
d=0.2 g, t=4 h	204	190	210
d=0.8 g, t=24 h	240	235	
d=0.8 g, t=24 h	187	173	214
d=0.5 g, t=24 h	228	214	
d=0.5 g, t=24 h	218	204	223
d=0.2 g, t=24 h	214	200	
d=0.2 g, t=24 h	191	177	203
d=0, t=24 h	168	154	
d=0, t=24 h	154	140	161

APPENDIX 8. KINETIC MINI-STUDY PART 1

Table A.41 Test run 1 colour measurement kinetic mini-study on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.016	0.017	0.017	
Std. 100	0.039	0.037	0.038	
Std. 300	0.100	0.102	0.101	
Std. 500	0.172	0.172	0.172	
t=0	0.116	0.115	0.116	
t=0	0.119	0.117	0.118	0.117
t=0.08 h	0.115	0.117	0.116	
t=0.08 h	0.114	0.111	0.113	0.114
t=0.17 h	0.108	0.108	0.108	
t=0.17 h	0.109	0.110	0.110	0.109
t=0.25 h	0.110	0.110	0.110	
t=0.25 h	0.115	0.116	0.116	0.113
t=0.5 h	0.106	0.108	0.107	
t=0.5 h	0.114	0.115	0.115	0.111
t=0.75 h	0.106	0.106	0.106	
t=0.75 h	0.107	0.108	0.108	0.107
t=1 h	0.102	0.102	0.102	
t=1 h	0.100	0.101	0.101	0.101
t=4 h	0.098	0.097	0.098	
t=4 h	0.101	0.100	0.101	0.099
t=8 h	0.089	0.089	0.089	
t=8 h	0.086	0.087	0.087	0.088
t=15 h	0.095	0.096	0.096	
t=15 h	0.094	0.095	0.095	0.095
t=19 h	0.088	0.087	0.088	
t=19 h	0.088	0.087	0.088	0.088
t=24 h	0.089	0.089	0.089	
t=24 h	0.089	0.089	0.089	0.089
t=32 h	0.086	0.083	0.085	
t=32 h	0.084	0.085	0.085	0.085
t=40 h	0.063	0.063	0.063	
t=40 h	0.061	0.063	0.062	0.063
t=44 h	0.076	0.077	0.077	
t=44 h	0.075	0.075	0.075	0.076
t=48 h	0.069	0.069	0.069	
t=48 h	0.073	0.072	0.073	0.071
Blank t=48 h	0.121	0.122	0.122	
Blank t=48 h	0.121	0.124	0.123	0.122
t=52 h	0.090	0.089	0.090	
t=52 h	0.091	0.095	0.093	0.091

Table A.42 Test run 1 COD measurement kinetic mini-study on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.023	0.023	0.023	
Std. 300	0.114	0.114	0.114	
Std. 500	0.193	0.192	0.193	
Std. 800	0.306	0.306	0.306	
t=0	0.113	0.113	0.113	
t=0	0.112	0.113	0.113	0.113
t=0.08 h	0.116	0.116	0.116	
t=0.08 h	0.117	0.117	0.117	0.117
t=0.17 h	0.118	0.117	0.118	
t=0.17 h	0.119	0.120	0.120	0.119
t=0.25 h	0.115	0.114	0.115	
t=0.25 h	0.120	0.119	0.120	0.117
t=0.5 h	0.110	0.110	0.110	
t=0.5 h	0.118	0.122	0.120	0.115
t=0.75 h	0.122	0.122	0.122	
t=0.75 h	0.121	0.122	0.122	0.122
t=1 h	0.122	0.120	0.121	
t=1 h	0.116	0.117	0.117	0.119
t=4 h	0.116	0.115	0.116	
t=4 h	0.118	0.118	0.118	0.117
t=8 h	0.112	0.112	0.112	
t=8 h	0.120	0.120	0.120	0.116
t=15 h	0.112	0.112	0.112	
t=15 h	0.122	0.123	0.123	0.117
t=19 h	0.117	0.117	0.117	
t=19 h	0.116	0.117	0.117	0.117
t=24 h	0.114	0.112	0.113	
t=24 h	0.113	0.113	0.113	0.113
t=32 h	0.111	0.112	0.112	
t=32 h	0.105	0.104	0.105	0.108
t=40 h	0.095	0.095	0.095	
t=40 h	0.100	0.100	0.100	0.098
t=44 h	0.101	0.101	0.101	
t=44 h	0.103	0.102	0.103	0.102
t=48 h	0.105	0.104	0.105	
t=48 h	0.115	0.116	0.116	0.110
Blank t=48 h	0.125	0.123	0.124	
Blank t=48 h	0.116	0.116	0.116	0.120
t=52 h	0.123	0.123	0.123	
t=52 h	0.126	0.126	0.126	0.125

Table A.43 Test run 1 DOC measurement kinetic mini-study on Western Pulp effluent

Sample	Height of peak (mm)				Value	Average
Blank	5	8	5	5	6	
Std. 50	16	15	17	17	16	
Std. 500	158	138	130	127	138	
t=0	67	66	67		67	
t=0	63	60	63		62	64
t=0.08 h	74	70	72	76	73	
t=0.08 h	72	71	74		72	73
t=0.17 h	70	70	71		70	
t=0.17 h	63	63	61		62	66
t=0.25 h	68	68	64		67	
t=0.25 h	66	62	66		65	66
t=0.5 h	66	69	67		67	
t=0.5 h	71	68	65		68	68
t=0.75 h	67	68	61		65	
t=0.75 h	71	75	75		74	70
t=1 h	76	74	69	79	75	
t=1 h	74	72	70	74	73	74
t=4 h	74	68	68		70	
t=4 h	70	78	68		72	71
t=8 h	68	70	65	68	68	
t=8 h	64	61	60		62	65
t=15 h	71	78	74	74	74	
t=15 h	72	71	70		71	73
t=19 h	63	64	63		63	
t=19 h	64	61	61		62	63
t=24 h	66	64	64		65	
t=24 h	68	68	68		68	66
t=32 h	59	59	56		58	
t=32 h	66	65	62		64	61
t=40 h	52	58	54		55	
t=40 h	56	54	58		56	55
t=44 h	53	54	52		53	
t=44 h	51	50	52		51	52
t=48 h	48	50	49		49	49
Blank t=48 h	56	62	67		62	
Blank t=48 h	62	63	63		63	62
t=52 h	63	67	66		65	
t=52 h	71	69	72	74	72	68

Table A.44 Test run 1 pH measurement kinetic mini-study on Western Pulp effluent

Sample	pH
t=0	8.15
t=0.08 h	8.38
t=0.17 h	8.46
t=0.25 h	8.20
t=0.50 h	8.32
t=0.75 h	8.49
t=1 h	8.55
t=4 h	8.41
t=8 h	8.12
t=15 h	8.92
t=19 h	9.03
t=24 h	8.89
t=32 h	8.63
t=40 h	8.36
t=44 h	8.56
t=48 h	8.49
Blank t=48 h	9.07
t=52hr	8.52

Table A.45 Test run 2 colour measurement kinetic mini-study on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.015	0.015	
Std. 100	0.037	0.037	0.037	
Std. 300	0.100	0.101	0.101	
Std. 500	0.176	0.176	0.176	
t=0	0.110	0.110	0.110	
t=0	0.112	0.111	0.112	0.111
t=0.08 h	0.106	0.105	0.106	
t=0.08 h	0.106	0.107	0.107	0.106
t=0.17 h	0.105	0.103	0.104	
t=0.17 h	0.103	0.104	0.104	0.104
t=0.25 h	0.106	0.106	0.106	
t=0.25 h	0.104	0.104	0.104	0.105
t=0.5 h	0.105	0.107	0.106	
t=0.5 h	0.106	0.106	0.106	0.106
t=0.75 h	0.099	0.098	0.099	
t=0.75 h	0.100	0.100	0.100	0.099
t=1 h	0.097	0.099	0.098	
t=1 h	0.099	0.100	0.100	0.099
t=4 h	0.091	0.093	0.092	
t=4 h	0.091	0.091	0.091	0.092
t=8 h	0.093	0.091	0.092	
t=8 h	0.092	0.092	0.092	0.092
t=15 h	0.084	0.084	0.084	
t=15 h	0.085	0.086	0.086	0.085
t=19 h	0.085	0.087	0.086	
t=19 h	0.084	0.081	0.083	0.084
t=24 h	0.082	0.080	0.081	
t=24 h	0.077	0.078	0.078	0.079
t=32 h	0.067	0.068	0.068	
t=32 h	0.067	0.066	0.067	0.067
t=40 h	0.072	0.072	0.072	
t=40 h	0.073	0.074	0.074	0.073
t=44 h	0.053	0.055	0.054	
t=44 h	0.053	0.052	0.053	0.053
t=48 h	0.064	0.065	0.065	
t=48 h	0.065	0.066	0.066	0.065
Blank t=48 h	0.115	0.114	0.115	
Blank t=48 h	0.115	0.112	0.114	0.114
t=52 h	0.048	0.049	0.049	
t=52 h	0.047	0.049	0.048	0.048

Table A.46 Test run 2 COD measurement kinetic mini-study on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.024	0.025	0.025	
Std. 300	0.115	0.115	0.115	
Std. 500	0.196	0.196	0.196	
Std. 800	0.310	0.310	0.310	
t=0	0.109	0.109	0.109	
t=0	0.115	0.115	0.115	0.112
t=0.08 h	0.111	0.112	0.112	
t=0.08 h	0.109	0.110	0.110	0.111
t=0.17 h	0.117	0.118	0.118	
t=0.17 h	0.108	0.109	0.109	0.113
t=0.25 h	0.110	0.112	0.111	
t=0.25 h	0.113	0.113	0.113	0.112
t=0.5 h	0.114	0.113	0.114	
t=0.5 h	0.118	0.119	0.119	0.116
t=0.75 h	0.116	0.118	0.117	
t=0.75 h	0.112	0.112	0.112	0.115
t=1 h	0.119	0.118	0.119	
t=1 h	0.112	0.112	0.112	0.115
t=4 h	0.115	0.115	0.115	
t=4 h	0.109	0.109	0.109	0.112
t=8 h	0.120	0.119	0.120	
t=8 h	0.119	0.119	0.119	0.119
t=15 h	0.120	0.120	0.120	
t=15 h	0.112	0.111	0.112	0.116
t=19 h	0.110	0.109	0.110	
t=19 h	0.115	0.114	0.115	0.112
t=24 h	0.104	0.104	0.104	
t=24 h	0.102	0.104	0.103	0.104
t=32 h	0.204	0.203	0.204	
t=32 h	0.202	0.200	0.201	0.202
t=40 h	0.111	0.111	0.111	
t=40 h	0.115	0.114	0.115	0.113
t=44 h	0.109	0.109	0.109	
t=44 h	0.119	0.118	0.119	0.114
t=48 h	0.116	0.116	0.116	
t=48 h	0.119	0.120	0.120	0.118
Blank t=48 h	0.123	0.121	0.122	
Blank t=48 h	0.122	0.121	0.122	0.122
t=52 h	0.091	0.090	0.091	
t=52 h	0.093	0.093	0.093	0.092

Table A.47 Test run 2 DOC measurement kinetic mini-study on Western Pulp effluent

Sample	Height of peak (mm)				Value	Average
Blank	4	5	5		5	
Std. 50	12	11	11		11	
Std. 500	102	96	90	95	96	
t=0	28	32	29		30	
t=0	37	36	36	35	36	33
t=0.08 h	26	36	34	35	33	
t=0.08 h	36	29	34	36	34	33
t=0.17 h	40	39	39		39	
t=0.17 h	41	40	24	41	37	38
t=0.25 h	41	40	39		40	
t=0.25 h	39	26	38	39	36	38
t=0.5 h	35	38	38	37	37	
t=0.5 h	39	33	38	40	38	37
t=0.75 h	38	39	22	38	34	
t=0.75 h	36	37	38		37	36
t=1 h	37	35	37	38	37	
t=1 h	37	38	36	38	37	37
t=4 h	33	37	36	36	36	
t=4 h	34	18	36	18	27	31
t=8 h	37	37	36		37	
t=8 h	22	36	35	19	28	32
t=15 h	34	29	35	35	33	
t=15 h	32	26	18	34	28	30
t=19 h	36	25	34		32	
t=19 h	35	35	12		27	30
t=24 h	34	33	32		33	
t=24 h	31	31	32		31	32
t=32 h	31	31	42		35	
t=32 h	31	38	34		34	35
t=40 h	34	32	34	43	36	
t=40 h	34	33	33		33	35
t=44 h	39	35	35	37	37	
t=44 h	37	38	37	37	37	37
t=48 h	33	31	31		32	
t=48 h	31	32	30		31	31
Blank t=48 h	32	31	32		32	
Blank t=48 h	32	31	33		32	32
t=52 h	32	27	32	32	31	
t=52 h	45	46	47		46	38

Table A.48 Test run 2 pH measurement kinetic mini-study on Western Pulp effluent

Sample	pH
t=0	7.83
t=0.08 h	8.28
t=0.17 h	8.34
t=0.25 h	8.16
t=0.50 h	8.36
t=0.75 h	8.39
t=1 h	8.24
t=4 h	8.29
t=8 h	8.81
t=15 h	8.96
t=19 h	9.02
t=24 h	8.61
t=32 h	8.54
t=40 h	8.40
t=44 h	8.45
t=48 h	8.56
Blank t=48 h	9.05
t=52hr	8.63

Table A.49 Colour measurement kinetic mini-study on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.016	0.018	0.017	
Std. 100	0.038	0.038	0.038	
Std. 300	0.104	0.103	0.104	
Std. 500	0.178	0.175	0.177	
t=0	0.073	0.074	0.074	
t=0	0.074	0.073	0.074	0.074
t=0.08 h	0.061	0.061	0.061	
t=0.08 h	0.060	0.061	0.061	0.061
t=0.17 h	0.062	0.061	0.062	
t=0.17 h	0.064	0.064	0.064	0.063
t=0.25 h	0.060	0.060	0.060	
t=0.25 h	0.059	0.061	0.060	0.060
t=0.5 h	0.067	0.066	0.067	
t=0.5 h	0.074	0.073	0.074	0.070
t=0.75 h	0.055	0.055	0.055	
t=0.75 h	0.059	0.058	0.059	0.057
t=1 h	0.050	0.053	0.052	
t=1 h	0.050	0.052	0.051	0.051
t=4 h	0.047	0.050	0.049	
t=4 h	0.045	0.046	0.046	0.047
t=8 h	0.045	0.044	0.045	
t=8 h	0.045	0.046	0.046	0.045
t=15 h	0.053	0.051	0.052	
t=15 h	0.052	0.052	0.052	0.052
t=19 h	0.054	0.054	0.054	
t=19 h	0.052	0.053	0.053	0.053
t=24 h	0.055	0.057	0.056	
t=24 h	0.053	0.052	0.053	0.054
t=32 h	0.043	0.043	0.043	
t=32 h	0.045	0.044	0.045	0.044
t=40 h	0.037	0.038	0.038	
t=40 h	0.038	0.038	0.038	0.038
t=44 h	0.041	0.040	0.041	
t=44 h	0.043	0.045	0.044	0.042
t=48 h	0.034	0.033	0.034	
t=48 h	0.037	0.037	0.037	0.035
Blank t=48 h	0.073	0.072	0.073	
Blank t=48 h	0.074	0.075	0.075	0.074
t=52 h	0.040	0.042	0.041	
t=52 h	0.043	0.043	0.043	0.042

Table A.50 COD measurement kinetic mini-study on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.040	0.038	0.039	
Std. 300	0.117	0.118	0.118	
Std. 500	0.202	0.202	0.202	
Std. 800	0.317	0.317	0.317	
t=0	0.099	0.096	0.098	
t=0	0.098	0.096	0.097	0.097
t=0.08 h	0.091	0.090	0.091	
t=0.08 h	0.089	0.089	0.089	0.090
t=0.17 h	0.097	0.097	0.097	
t=0.17 h	0.097	0.097	0.097	0.097
t=0.25 h	0.087	0.088	0.088	
t=0.25 h	0.091	0.092	0.092	0.090
t=0.5 h	0.109	0.109	0.109	
t=0.5 h	0.140	0.140	0.140	0.125
t=0.75 h	0.099	0.099	0.099	
t=0.75 h	0.128	0.128	0.128	0.114
t=1 h	0.096	0.095	0.096	
t=1 h	0.088	0.089	0.089	0.092
t=4 h	0.093	0.094	0.094	
t=4 h	0.092	0.090	0.091	0.092
t=8 h	0.078	0.077	0.078	
t=8 h	0.077	0.077	0.077	0.077
t=15 h	0.095	0.095	0.095	
t=15 h	0.097	0.096	0.097	0.096
t=19 h	0.100	0.100	0.100	
t=19 h	0.111	0.111	0.111	0.106
t=24 h	0.102	0.100	0.101	
t=24 h	0.129	0.126	0.128	0.114
t=32 h	0.090	0.089	0.090	
t=32 h	0.106	0.106	0.106	0.098
t=40 h	0.086	0.085	0.086	
t=40 h	0.082	0.082	0.082	0.084
t=44 h	0.093	0.093	0.093	
t=44 h	0.081	0.082	0.082	0.087
t=48 h	0.081	0.082	0.082	
t=48 h	0.082	0.082	0.082	0.082
Blank t=48 h	0.102	0.101	0.102	
Blank t=48 h	0.112	0.114	0.113	0.107
t=52 h	0.096	0.096	0.096	
t=52 h	0.099	0.099	0.099	0.098

Table A.51 DOC measurement kinetic mini-study on Howe Sound effluent

Standard	Height of peak (mm)				Value	Average
Blank	4	4	4		4	
Std. 50	19	18	16		18	
Std. 500	184	179	163	171	174	
t=0	54	51	52		52	
t=0	44	46	45		45	49
t=0.08 h	52	50	49		50	
t=0.08 h	51	52	50		51	51
t=0.17 h	51	56	54		54	
t=0.17 h	52	53	54		53	53
t=0.25 h	52	51	51		51	
t=0.25 h	54	52	54		53	52
t=0.5 h	56	54	55		55	
t=0.5 h	60	64	62		62	59
t=0.75 h	54	51	52		52	
t=0.75 h	51	51	54		52	52
t=1 h	49	50	49		49	
t=1 h	47	47	46		47	48
t=4 h	48	47	47		47	
t=4 h	47	49	46		47	47
t=8 h	47	48	47		47	
t=8 h	47	45	47		46	47
t=15 h	54	52	53		53	
t=15 h	51	52	50		51	52
t=19 h	52	51	52		52	
t=19 h	48	49	48	46	48	50
t=24 h	49	50	49		49	
t=24 h	50	55	52	52	52	51
t=32 h	51	47	50		49	
t=32 h	48	45	49	47	47	48
t=40 h	48	47	49		48	
t=40 h	45	48	44		46	47
t=44 h	41	40	39		40	
t=44 h	38	42	40		40	40
t=48 h	41	40	40		40	
t=48 h	44	45	44		44	42
Blank t=48 h	46	46	47	47	47	
Blank t=48 h	41	40	40		40	43
t=52 h	46	47	46		46	
t=52 h	52	53	52		52	49

Table A.52 pH measurement kinetic mini-study on Howe Sound effluent

Sample	pH
t=0	7.59
t=0.08 h	8.35
t=0.17 h	8.31
t=0.25 h	8.26
t=0.50 h	8.28
t=0.75 h	8.33
t=1 h	8.57
t=4 h	8.33
t=8 h	8.35
t=15 h	8.92
t=19 h	8.88
t=24 h	8.75
t=32 h	8.51
t=40 h	8.41
t=44 h	8.57
t=48 h	8.60
Blank t=48 h	9.06
t=52hr	8.60

APPENDIX 9. INHIBITION MINI-STUDY AND KINETIC MINI-STUDY PART 2

Table A.53 Test run 1 colour measurement kinetic rate at 4°C on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.014	0.014	0.014	
Std. 100	0.038	0.038	0.038	
Std. 300	0.101	0.102	0.102	
Std. 500	0.175	0.177	0.176	
t=0	0.124	0.124	0.124	
t=0	0.121	0.120	0.121	0.122
t=1 h	0.105	0.105	0.105	
t=1 h	0.107	0.106	0.107	0.106
t=8 h	0.102	0.103	0.103	
t=8 h	0.103	0.101	0.102	0.102
t=19 h	0.095	0.096	0.096	
t=19 h	0.095	0.093	0.094	0.095
t=24 h	0.096	0.095	0.096	
t=24 h	0.093	0.094	0.094	0.095
t=32 h	0.092	0.095	0.094	
t=32 h	0.093	0.095	0.094	0.094
t=40 h	0.090	0.089	0.090	
t=40 h	0.089	0.084	0.087	0.088
t=48 h	0.088	0.087	0.088	
t=48 h	0.089	0.089	0.089	0.088
Blank t=48 h	0.121	0.118	0.120	
Blank t=48 h	0.117	0.117	0.117	0.118
t=52 h	0.091	0.090	0.091	
t=52 h	0.093	0.093	0.093	0.092

Table A.54 Test run 1 COD measurement kinetic rate at 4°C on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.014	0.014	0.014	
Std. 300	0.038	0.038	0.038	
Std. 500	0.101	0.102	0.102	
Std. 800	0.175	0.177	0.176	
t=0	0.124	0.124	0.124	
t=0	0.121	0.120	0.121	0.122
t=1 h	0.105	0.105	0.105	
t=1 h	0.107	0.106	0.107	0.106
t=8 h	0.102	0.103	0.103	
t=8 h	0.103	0.101	0.102	0.102
t=19 h	0.095	0.096	0.096	
t=19 h	0.095	0.093	0.094	0.095
t=24 h	0.096	0.095	0.096	
t=24 h	0.093	0.094	0.094	0.095
t=32 h	0.092	0.095	0.094	
t=32 h	0.093	0.095	0.094	0.094
t=40 h	0.09	0.089	0.090	
t=40 h	0.089	0.084	0.087	0.088
t=48 h	0.088	0.087	0.088	
t=48 h	0.089	0.089	0.089	0.088
Blank t=48 h	0.121	0.118	0.120	
Blank t=48 h	0.117	0.117	0.117	0.118
t=52 h	0.091	0.090	0.091	
t=52 h	0.093	0.093	0.093	0.092

Table A.55 Test run 1 DOC measurement kinetic rate at 4°C on Western Pulp effluent

Sample	Height of peak (mm)				Value	Average
Blank	6	6	2	5	5	
Std. 50	19	18	18		18	
Std. 500	178	187	174		180	
t=0	44	74	72	74	66	
t=0	73	73	74		73	70
t=1 h	76	89	73	76	79	
t=1 h	78	77	78		78	78
t=8 h	74	77	77		76	
t=8 h	75	73	77		75	76
t=19 h	68	65	69		67	
t=19 h	73	70	70		71	69
t=24 h	60	62	57	58	59	
t=24 h	62	64	65	68	65	62
t=32 h	58	57	61		59	
t=32 h	56	55	56		56	57
t=40 h	58	56	57		57	
t=40 h	62	59	64	67	63	60
t=48 h	48	62	64	67	60	
t=48 h	56	56	54		55	58
Blank t=48 h	62	58	62		61	
Blank t=48 h	69	54	66	66	64	62
t=52 h	58	57	57		57	
t=52 h	56	55	57		56	57

Table A.56 Test run 1 pH measurement kinetic rate at 4°C on Western Pulp effluent

Sample	pH
t=0	7.55
t=1 h	8.38
t=8 h	8.46
t=19 h	8.48
t=24 h	8.52
t=32 h	8.59
t=40 h	8.61
t=48 h	8.66
Blank t=48 h	8.68
t=52 h	8.58

Table A.57 Test run 2 colour measurement kinetic rate at 4°C on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.016	0.018	0.017	
Std. 100	0.038	0.038	0.038	
Std. 300	0.104	0.103	0.104	
Std. 500	0.178	0.175	0.177	
t=0	0.123	0.122	0.123	
t=0	0.123	0.124	0.124	0.123
t=1 h	0.101	0.103	0.102	
t=1 h	0.100	0.100	0.100	0.101
t=8 h	0.091	0.093	0.092	
t=8 h	0.092	0.092	0.092	0.092
t=19 h	0.088	0.087	0.088	
t=19 h	0.090	0.088	0.089	0.088
t=24 h	0.087	0.089	0.088	
t=24 h	0.089	0.087	0.088	0.088
t=32 h	0.084	0.083	0.084	
t=32 h	0.079	0.084	0.082	0.083
t=40 h	0.101	0.097	0.099	
t=40 h	0.103	0.102	0.103	0.101
t=48 h	0.095	0.093	0.094	
t=48 h	0.089	0.090	0.090	0.092
Blank t=48hr	0.131	0.126	0.129	
Blank t=48hr	0.119	0.116	0.118	0.123
t=52 h	0.084	0.081	0.083	
t=52 h	0.081	0.079	0.080	0.081

Table A.58 Test run 2 COD measurement kinetic rate at 4°C on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.040	0.038	0.039	
Std. 300	0.117	0.118	0.118	
Std. 500	0.202	0.202	0.202	
Std. 800	0.317	0.317	0.317	
t=0	0.120	0.120	0.120	
t=0	0.127	0.126	0.127	0.123
t=1 h	0.133	0.132	0.133	
t=1 h	0.119	0.117	0.118	0.125
t=8 h	0.108	0.108	0.108	
t=8 h	0.115	0.115	0.115	0.112
t=19 h	0.115	0.115	0.115	
t=19 h	0.113	0.113	0.113	0.114
t=24 h	0.120	0.120	0.120	
t=24 h	0.121	0.122	0.122	0.121
t=32 h	0.104	0.104	0.104	
t=32 h	0.096	0.093	0.095	0.099
t=40 h	0.111	0.112	0.112	
t=40 h	0.116	0.115	0.116	0.114
t=48 h	0.113	0.113	0.113	
t=48 h	0.116	0.114	0.115	0.114
Blank t=48 h	0.120	0.121	0.121	
Blank t=48 h	0.119	0.118	0.119	0.120
t=52 h	0.113	0.113	0.113	
t=52 h	0.119	0.118	0.119	0.116

Table A.59 Test run 2 DOC measurement kinetic rate at 4°C on Western Pulp effluent

Sample	Height of peak (mm)				Value	Average
Blank	4	4	4		4	
Std. 50	19	18	16		18	
Std. 500	184	179	163	171	174	
t=0	64	65	63		64	
t=0	69	66	63	67	66	65
t=1 h	70	70	73		71	
t=1 h	71	71	71		71	71
t=8 h	70	73	71		71	
t=8 h	70	66	66		67	69
t=19 h	58	57	58		58	
t=19 h	56	58	57		57	57
t=24 h	70	69	70		70	
t=24 h	55	58	57		57	63
t=32 h	51	50	47		49	
t=32 h	51	52	53		52	51
t=40 h	47	54	54	53	52	
t=40 h	56	55	55		55	54
t=48 h	52	50	49	51	51	
t=48 h	51	54	52		52	51
Blank t=48 h	52	50	52		51	
Blank t=48 h	53	55	56		55	53
t=52 h	52	52	57		54	
t=52 h	48	47	48		48	51

Table A.60 Test run 2 pH measurement kinetic rate at 4°C on Western Pulp effluent

Sample	pH
t=0	7.50
t=1 h	8.27
t=8 h	8.39
t=19 h	8.57
t=24 h	8.67
t=32 h	8.40
t=40 h	8.64
t=48 h	8.70
Blank t=48 h	8.71
t=52 h	8.60

Table A.61 Colour measurement kinetic rate at 4°C on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.016	0.016	
Std. 100	0.039	0.038	0.039	
Std. 300	0.101	0.102	0.102	
Std. 500	0.173	0.172	0.173	
t=0	0.077	0.076	0.077	
t=0	0.073	0.073	0.073	0.075
t=1 h	0.055	0.056	0.056	
t=1 h	0.055	0.055	0.055	0.055
t=8 h	0.054	0.054	0.054	
t=8 h	0.052	0.052	0.052	0.053
t=19 h	0.048	0.049	0.049	
t=19 h	0.050	0.050	0.050	0.049
t=24 h	0.049	0.048	0.049	
t=24 h	0.048	0.047	0.048	0.048
t=32 h	0.051	0.049	0.050	
t=32 h	0.049	0.05	0.050	0.050
t=40 h	0.046	0.047	0.047	
t=40 h	0.043	0.045	0.044	0.045
t=48 h	0.046	0.045	0.046	
t=48 h	0.045	0.045	0.045	0.045
Blank t=48hr	0.076	0.074	0.075	
Blank t=48hr	0.074	0.074	0.074	0.075
t=52 h	0.052	0.052	0.052	
t=52 h	0.055	0.054	0.055	0.053

Table A.62 COD measurement kinetic rate at 4°C on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.038	0.038	0.038	
Std. 300	0.117	0.116	0.117	
Std. 500	0.200	0.201	0.201	
Std. 800	0.316	0.316	0.316	
t=0	0.088	0.089	0.089	
t=0	0.089	0.090	0.090	0.089
t=1 h	0.084	0.085	0.085	
t=1 h	0.089	0.089	0.089	0.087
t=8 h	0.095	0.095	0.095	
t=8 h	0.103	0.104	0.104	0.099
t=19 h	0.084	0.084	0.084	
t=19 h	0.083	0.083	0.083	0.084
t=24 h	0.098	0.098	0.098	
t=24 h	0.090	0.089	0.090	0.094
t=32 h	0.086	0.086	0.086	
t=32 h	0.089	0.089	0.089	0.088
t=40 h	0.093	0.093	0.093	
t=40 h	0.095	0.095	0.095	0.094
t=48 h	0.095	0.092	0.094	
t=48 h	0.093	0.093	0.093	0.093
Blank t=48 h	0.101	0.101	0.101	
Blank t=48 h	0.107	0.105	0.106	0.104
t=52 h	0.106	0.106	0.106	
t=52 h	0.102	0.100	0.101	0.104

Table A.63 DOC measurement kinetic rate at 4°C on Howe Sound effluent

Standard	Height of peak (mm)				Value	Average
Blank	5	5	5		5	
Std. 50	16	18	17		17	
Std. 500	175	176	168	171	173	
t=0	55	58	57		57	
t=0	56	59	63		59	58
t=1 h	57	54	55		55	
t=1 h	55	54	58		56	56
t=8 h	55	54	52		54	
t=8 h	49	53	49		50	52
t=19 h	45	50	52		49	
t=19 h	53	51	52		52	51
t=24 h	43	43	45		44	
t=24 h	51	52	51		51	48
t=32 h	47	48	46	45	47	
t=32 h	43	43	41		42	44
t=40 h	54	50	51		52	
t=40 h	55	51	57		54	53
t=48 h	47	49	47		48	
t=48 h	54	49	51		51	50
Blank t=48 h	58	55	54		56	
Blank t=48 h	58	57	52		56	56
t=52 h	50	52	59		54	
t=52 h	53	51	52		52	53

Table A.64 pH measurement kinetic rate at 4°C on Howe Sound effluent

Sample	pH
t=0	7.57
t=1 h	8.21
t=8 h	8.50
t=19 h	8.78
t=24 h	8.50
t=32 h	8.52
t=40 h	8.58
t=48 h	8.72
Blank t=48 h	8.76
t=52 h	8.52

Table A.65 Colour measurement kinetic rate with NaN_3 addition at room temperature on Western Pulp effluent

Sample	Absorbance			Value	Average
Blank	0			0	
Std. 50	0.014	0.017		0.016	
Std. 100	0.040	0.039		0.040	
Std. 300	0.103	0.103		0.103	
Std. 500	0.174	0.174		0.174	
t=0	0.123	0.124		0.124	
t=0	0.118	0.120		0.119	0.121
t=1 h	0.101	0.102		0.102	
t=1 h	0.097	0.099		0.098	0.100
t=8 h	0.091	0.091	0.093	0.091	
t=8 h	0.088	0.088	0.091	0.088	0.090
t=24 h	0.087	0.085		0.086	
t=24 h	0.083	0.085		0.084	0.085
t=32 h	0.080	0.081		0.081	
t=32 h	0.081	0.080		0.081	0.081
t=40 h	0.079	0.080		0.080	
t=40 h	0.082	0.083		0.083	0.081
t=48 h	0.082	0.077	0.081	0.080	
t=48 h	0.076	0.077		0.077	0.078
Blank t=48 h	0.121	0.120		0.121	
Blank t=48 h	0.123	0.124		0.124	0.122

Table A.66 COD measurement kinetic rate with NaN_3 addition at room temperature on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.038	0.038	0.038	
Std. 300	0.117	0.118	0.118	
Std. 500	0.201	0.201	0.201	
Std. 800	0.318	0.318	0.318	
t=0	0.122	0.122	0.122	
t=0	0.120	0.120	0.120	0.121
t=1 h	0.121	0.122	0.122	
t=1 h	0.123	0.124	0.124	0.123
t=8 h	0.119	0.120	0.120	
t=8 h	0.120	0.120	0.120	0.120
t=24 h	0.122	0.119	0.121	
t=24 h	0.120	0.121	0.121	0.121
t=32 h	0.119	0.118	0.119	
t=32 h	0.128	0.128	0.128	0.123
t=40 h	0.119	0.120	0.120	
t=40 h	0.124	0.126	0.125	0.122
t=48 h	0.120	0.119	0.120	
t=48 h	0.120	0.120	0.120	0.120
Blank t=48 h	0.149	0.152	0.151	
Blank t=48 h	0.137	0.137	0.137	0.144

Table A.67 DOC measurement kinetic rate with NaN_3 addition at room temperature on Western Pulp effluent

Sample	Height of peak (mm)				Value	Average
Blank	5	5	5		5	
Std. 50	16	16	15		16	
Std. 500	169	161	164		165	
t=0	75	74	76		75	
t=0	79	74	72		75	75
t=1 h	82	79	80		80	
t=1 h	70	71	73	75	72	76
t=8 h	78	80	82		80	
t=8 h	82	86	84		84	82
t=24 h	75	71	75		74	
t=24 h	69	69	73		70	72
t=32 h	72	83	77		77	
t=32 h	75	75	78		76	77
t=40 h	65	67	61		64	
t=40 h	61	59	60		60	62
t=48 h	65	65	71		67	
t=48 h	58	60	62		60	64
Blank t=48 h	69	73	75		72	
Blank t=48 h	67	68	70		68	70

Table A.68 pH measurement kinetic rate with NaN_3 addition at room temperature on Western Pulp effluent

Sample	pH
t=0	7.72
t=1 h	8.35
t=8 h	8.88
t=24 h	8.93
t=32 h	8.90
t=40 h	8.73
t=48 h	8.79
Blank t=48 h	9.05

Table A.69 Colour measurement kinetic rate with NaN_3 addition at room temperature on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.014	0.015	
Std. 100	0.037	0.039	0.038	
Std. 300	0.102	0.103	0.103	
Std. 500	0.173	0.173	0.173	
t=0	0.070	0.071	0.071	
t=0	0.073	0.071	0.072	0.071
t=1 h	0.051	0.051	0.051	
t=1 h	0.051	0.050	0.051	0.051
t=8 h	0.051	0.050	0.051	
t=8 h	0.053	0.053	0.053	0.052
t=24 h	0.052	0.053	0.053	
t=24 h	0.055	0.054	0.055	0.054
t=32 h	0.047	0.047	0.047	
t=32 h	0.048	0.047	0.048	0.047
t=40 h	0.047	0.05	0.049	
t=40 h	0.045	0.045	0.045	0.047
t=48 h	0.038	0.038	0.038	
t=48 h	0.039	0.041	0.040	0.039
Blank t=48 h	0.078	0.077	0.078	
Blank t=48 h	0.076	0.078	0.077	0.077

Table A.70 COD measurement kinetic rate with NaN_3 addition at room temperature on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.038	0.038	0.038	
Std. 300	0.117	0.116	0.117	
Std. 500	0.199	0.200	0.200	
Std. 800	0.316	0.315	0.316	
t=0	0.091	0.091	0.091	
t=0	0.092	0.093	0.093	0.092
t=1 h	0.084	0.083	0.084	
t=1 h	0.086	0.085	0.086	0.085
t=8 h	0.100	0.100	0.100	
t=8 h	0.098	0.098	0.098	0.099
t=24 h	0.105	0.105	0.105	
t=24 h	0.106	0.108	0.107	0.106
t=32 h	0.098	0.097	0.098	
t=32 h	0.099	0.098	0.099	0.098
t=40 h	0.100	0.100	0.100	
t=40 h	0.099	0.100	0.100	0.100
t=48 h	0.085	0.085	0.085	
t=48 h	0.087	0.086	0.087	0.086
Blank t=48 h	0.104	0.104	0.104	
Blank t=48 h	0.107	0.107	0.107	0.106

Table A.71 DOC measurement kinetic rate with NaN_3 addition at room temperature on Howe Sound effluent

Standard	Height of peak (mm)				Average
Blank	3	3	2		3
Std. 50	11	10	10		10
Std. 500	106	105	100	171	121
t=0	31	31	32	31	
t=0	31	30	31	31	31
t=1 h	30	31	30	30	
t=1 h	29	31	30	30	30
t=8 h	34	34	33	34	
t=8 h	32	32	32	32	33
t=24 h	36	36	35	36	
t=24 h	35	36	37	36	36
t=32 h	24	25	26	25	
t=32 h	32	32	31	32	28
t=40 h	25	25	25	25	
t=40 h	29	30	31	30	28
t=48 h	28	28	28	28	
t=48 h	28	30	28	29	28
Blank t=48 h	31	32	31	31	
Blank t=48 h	29	28	28	28	30

Table A.72 pH measurement kinetic rate with NaN_3 addition at room temperature on Howe Sound effluent

Sample	pH
t=0	7.72
t=1 h	8.35
t=8 h	8.88
t=24 h	8.93
t=32 h	8.90
t=40 h	8.73
t=48 h	8.79
Blank t=48 h	9.05

Table A.73 Colour measurement kinetic rate with NaF addition at room temperature on Western Pulp effluent

Sample	Absorbance			Average
Blank	0		0	
Std. 50	0.014	0.017	0.016	
Std. 100	0.040	0.039	0.040	
Std. 300	0.103	0.103	0.103	
Std. 500	0.174	0.174	0.174	
t=0	0.120	0.123	0.122	
t=0	0.124	0.120	0.122	0.122
t=1 h	0.103	0.101	0.102	
t=1 h	0.103	0.101	0.102	0.102
t=8 h	0.091	0.089	0.090	
t=8 h	0.090	0.089	0.090	0.090
t=24 h	0.083	0.083	0.083	
t=24 h	0.084	0.085	0.085	0.084
t=32 h	0.070	0.069	0.070	
t=32 h	0.073	0.073	0.073	0.071
t=40 h	0.069	0.070	0.070	
t=40 h	0.070	0.069	0.070	0.070
t=48 h	0.073	0.071	0.072	
t=48 h	0.076	0.075	0.076	0.074
Blank t=48 h	0.113	0.115	0.114	
Blank t=48 h	0.118	0.118	0.118	0.116

Table A.74 COD measurement kinetic rate with NaF addition at room temperature on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.038	0.038	0.038	
Std. 300	0.117	0.118	0.118	
Std. 500	0.201	0.201	0.201	
Std. 800	0.318	0.318	0.318	
t=0	0.125	0.125	0.125	
t=0	0.115	0.116	0.116	0.120
t=1 h	0.118	0.118	0.118	
t=1 h	0.118	0.118	0.118	0.118
t=8 h	0.112	0.112	0.112	
t=8 h	0.117	0.115	0.116	0.114
t=24 h	0.111	0.111	0.111	
t=24 h	0.115	0.116	0.116	0.113
t=32 h	0.101	0.101	0.101	
t=32 h	0.101	0.101	0.101	0.101
t=40 h	0.110	0.110	0.110	
t=40 h	0.119	0.117	0.118	0.114
t=48 h	0.117	0.119	0.118	
t=48 h	0.124	0.126	0.125	0.122
Blank t=48 h	0.126	0.128	0.127	
Blank t=48 h	0.142	0.142	0.142	0.127

Table A.75 DOC measurement kinetic rate with NaF addition at room temperature on Western Pulp effluent

Sample	Height of peak (mm)				Average
Blank	3	3	2		3
Std. 50	11	10	10		10
Std. 500	106	105	100	171	121
t=0	31	31	32	31	
t=0	31	30	31	31	31
t=1 h	30	31	30	30	
t=1 h	29	31	30	30	30
t=8 h	34	34	33	34	
t=8 h	32	32	32	32	33
t=24 h	36	36	35	36	
t=24 h	35	36	37	36	36
t=32 h	24	25	26	25	
t=32 h	32	32	31	32	28
t=40 h	25	25	25	25	
t=40 h	29	30	31	30	28
t=48 h	28	28	28	28	
t=48 h	28	30	28	29	28
Blank t=48 h	31	32	31	31	
Blank t=48 h	29	28	28	28	30

Table A.76 pH measurement kinetic rate with NaN_3 addition at room temperature on Howe Sound effluent

Sample	pH
t=0	7.81
t=1 h	8.49
t=8 h	8.60
t=24 h	8.98
t=32 h	9.06
t=40 h	8.59
t=48 h	8.66
Blank t=48 h	9.05

APPENDIX 10. ISOTHERM MINI-STUDY

Table A.77 Colour measurement equilibrium isotherms with NaN_3 addition at room temperature on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.014	0.015	
Std. 100	0.037	0.036	0.037	
Std. 300	0.099	0.100	0.100	
Std. 500	0.167	0.169	0.168	
d=0, t=0	0.118	0.118	0.118	
d=0, t=0	0.118	0.115	0.117	0.117
d=0.1 g, t=32 h	0.093	0.091	0.092	
d=0.1 g, t=32 h	0.089	0.088	0.089	0.090
d=0.5 g, t=32 h	0.063	0.061	0.062	
d=0.5 g, t=32 h	0.061	0.061	0.061	0.062
d=1.0 g, t=32 h	0.060	0.058	0.059	
d=1.0 g, t=32 h	0.054	0.050	0.052	0.056
d=2.0 g, t=32 h	0.039	0.038	0.039	
d=2.0 g, t=32 h	0.043	0.041	0.042	0.040
d=3.0 g, t=32 h	0.045	0.045	0.045	
d=3.0 g, t=32 h	0.045	0.044	0.045	0.045
d=0, t=32 h	0.107	0.108	0.108	
d=0, t=32 h	0.110	0.108	0.109	0.108

Table A.78 COD measurement equilibrium isotherms with NaN_3 addition at room temperature on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.035	0.036	0.036	
Std. 300	0.121	0.122	0.122	
Std. 500	0.190	0.192	0.191	
Std. 800	0.274	0.276	0.275	
d=0, t=0	0.101	0.101	0.101	
d=0, t=0	0.100	0.099	0.100	0.100
d=0.1 g, t=32 h	0.120	0.119	0.120	
d=0.1 g, t=32 h	0.120	0.119	0.120	0.120
d=0.5 g, t=32 h	0.133	0.133	0.133	
d=0.5 g, t=32 h	0.141	0.142	0.142	0.137
d=2.0 g, t=32 h	0.319	0.319	0.319	
d=2.0 g, t=32 h	0.330	0.332	0.331	0.325
d=3.0 g, t=32 h	0.494	0.495	0.495	
d=3.0 g, t=32 h	0.481	0.478	0.480	0.487

Table A.79 DOC measurement equilibrium isotherms with NaN_3 addition at room temperature on Western Pulp effluent

Sample	Height of peak (mm)				Value	Average
Blank		1	1	1		1
Std. 50		11	10	10		10
Std. 500		93	94	98	105	98
d=0, t=0	36	35	36		36	36
d=0.1 g, t=32 h	34	35	36	36	35	35
d=0.5 g, t=32 h	36	37	37		37	37
d=1.0 g, t=32 h	29	29	31		30	
d=1.0 g, t=32 h	32	31	33		32	31
d=2.0 g, t=32 h	113	120	122	119	119	119
d=3.0 g, t=32 h	173	172	169	165	170	170
d=0, t=32 h	24	24	24		24	
d=0, t=32 h	23	23	23		23	24

Table A.80 pH measurement equilibrium isotherms with NaN_3 addition at room temperature on Western Pulp effluent

Sample	pH
d=0, t=0	8.07
d=0.1 g, t=32 h	8.79
d=0.5 g, t=32 h	8.60
d=2.0 g, t=32 h	8.34
d=3.0 g, t=32 h	8.49

Table A.81 Colour measurement equilibrium isotherms with NaN_3 addition at room temperature on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.014	0.015	
Std. 100	0.037	0.036	0.037	
Std. 300	0.099	0.100	0.100	
Std. 500	0.167	0.169	0.168	
d=0, t=0	0.078	0.079	0.079	
d=0, t=0	0.080	0.083	0.082	0.080
d=0.1 g, t=32 h	0.054	0.053	0.054	
d=0.1 g, t=32 h	0.059	0.058	0.059	0.056
d=0.5 g, t=32 h	0.036	0.036	0.036	
d=0.5 g, t=32 h	0.034	0.036	0.035	0.036
d=1.0 g, t=32 h	0.022	0.024	0.023	
d=1.0 g, t=32 h	0.018	0.018	0.018	0.021
d=2.0 g, t=32 h	0.020	0.022	0.021	
d=2.0 g, t=32 h	0.018	0.016	0.017	0.019
d=3.0 g, t=32 h	0.022	0.020	0.021	
d=3.0 g, t=32 h	0.019	0.020	0.020	0.020
d=0, t=32 h	0.081	0.080	0.081	
d=0, t=32 h	0.082	0.081	0.082	0.081

Table A.82 COD measurement equilibrium isotherms with NaN_3 addition at room temperature on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.035	0.036	0.036	
Std. 300	0.121	0.122	0.122	
Std. 500	0.190	0.192	0.191	
Std. 800	0.274	0.276	0.275	
d=0, t=0	0.116	0.115	0.116	
d=0, t=0	0.114	0.114	0.114	0.115
d=0.1 g, t=32 h	0.093	0.092	0.093	
d=0.1 g, t=32 h	0.093	0.093	0.093	0.093
d=0.5 g, t=32 h	0.095	0.095	0.095	
d=0.5 g, t=32 h	0.098	0.098	0.098	0.097
d=2.0 g, t=32 h	0.296	0.297	0.297	
d=2.0 g, t=32 h	0.264	0.266	0.265	0.281
d=3.0 g, t=32 h	0.317	0.316	0.317	
d=3.0 g, t=32 h	0.319	0.32	0.320	0.318

Table A.83 DOC measurement equilibrium isotherms with NaN_3 addition at room temperature on Howe Sound effluent

Sample	Height of peak (mm)				Value	Average
Blank	1	1	1		1	
Std. 50	11	10	10		10	
Std. 500	93	94	98	105	98	
d=0, t=0	23	24	24		24	24
d=0.1 g, t=32 h	27	27	29		28	28
d=0.5 g, t=32 h	26	27	26		26	26
d=1.0 g, t=32 h	15	15	15		15	
d=1.0 g, t=32 h	14	14	14		14	15
d=2.0 g, t=32 h	74	76	75		75	75
d=3.0 g, t=32 h	139	135	138	134	137	137
d=0, t=32 h	30	29	31		30	
d=0, t=32 h	34	34	35		34	32

Table A.84 pH measurement equilibrium isotherms with NaN_3 addition at room temperature on Howe Sound effluent

Sample	pH
d=0, t=0	7.81
d=0.1 g, t=32 h	8.56
d=0.5 g, t=32 h	8.50
d=2.0 g, t=32 h	8.20
d=3.0 g, t=32 h	8.30

APPENDIX 11. REMOVED FRACTION MINI-STUDY

Table A.85 Colour measurement of 1g dose of biomass for removed fraction mini-study on Western Pulp effluent

Sample	Absorbance			Value	Average
Blank	0				
Std. 50	0.015	0.014		0.015	
Std. 100	0.037	0.036		0.037	
Std. 300	0.099	0.100		0.100	
Std. 500	0.167	0.169		0.168	
500 Daltons	0.005	0.005	0.005		
500 Daltons	0.003	0.003	0.003	0.004	0.008
3000 Daltons	0.006	0.006	0.006		
3000 Daltons	0.006	0.006	0.006	0.006	0.012
10000 Daltons	0.008	0.008	0.008		
10000 Daltons	0.012	0.011	0.012	0.010	0.02
100000 Daltons	0.019	0.019	0.019		
100000 Daltons	0.017	0.018	0.018	0.018	0.036
Total	0.027	0.025	0.026		
Total	0.03	0.029	0.030	0.028	0.056

Table A.86 TOC measurement of 1g dose of biomass for removed fraction mini-study on Western Pulp effluent

Sample	Peak height			Average
3000 Daltons	6	6	6	
3000 Daltons	6	6	6	6
10000 Daltons	9	8	9	
10000 Daltons	9	9	9	9
100000 Daltons	12	13	13	
100000 Daltons	14	14	13	13
Total	28	29	28	
Total	29	29	29	29

Table A.87 Colour measurement of blank for removed fraction mini-study on Western Pulp effluent

Sample	Absorbance			Average
Blank	0		0	
Std. 50	0.015	0.014	0.015	
Std. 100	0.037	0.036	0.037	
Std. 300	0.099	0.100	0.100	
Std. 500	0.167	0.169	0.168	
500 Daltons	0.005	0.006	0.006	
500 Daltons	0.004	0.004	0.004	0.005
3000 Daltons	0.013	0.013	0.013	
3000 Daltons	0.014	0.013	0.014	0.013
10000 Daltons	0.028	0.030	0.029	
10000 Daltons	0.026	0.027	0.027	0.028
100000 Daltons	0.096	0.097	0.097	
100000 Daltons	0.094	0.093	0.094	0.095
Total	0.110	0.108	0.109	
Total	0.107	0.108	0.108	0.108

Table A.88 COD measurement of blank for removed fraction mini-study on Western Pulp effluent

Sample	Peak height			Average
500 Daltons	8	8	8	
500 Daltons	8	8	8	8
3000 Daltons	12	12	12	
3000 Daltons	12	12	12	12
10000 Daltons	17	16	17	
10000 Daltons	16	16	17	17
100000 Daltons	28	28	29	
100000 Daltons	30	29	29	29
Total	32	31	33	
Total	29	29	31	31

Table A.89 Colour measurement of 1g dose of biomass for removed fraction mini-study on Howe Sound effluent

Sample	Absorbance			Average	Adjusted as per dilution
Blank	0			0	
Std. 50	0.015	0.014		0.015	
Std. 100	0.037	0.036		0.037	
Std. 300	0.099	0.100		0.100	
Std. 500	0.167	0.169		0.168	
500 Daltons	0.006	0.006		0.006	
500 Daltons	0.005	0.005	0.005	0.006	0.012
3000 Daltons	0.011	0.01	0.011		
3000 Daltons	0.009	0.006	0.008	0.009	0.018
10000 Daltons	0.008	0.008	0.008		
10000 Daltons	0.008	0.008	0.008	0.008	0.016
100000 Daltons	0.009	0.009	0.009		
100000 Daltons	0.010	0.011	0.011	0.010	0.020
Total	0.009	0.009	0.009		
Total	0.011	0.012	0.012	0.010	0.020

Table A.90 TOC measurement of 1g dose of biomass for removed fraction mini-study on Howe Sound effluent

Sample	Peak height (mm)			Value
3000 Daltons	8	8	8	
3000 Daltons	8	8	8	8
10000 Daltons	9	8	9	
10000 Daltons	9	9	9	9
100000 Daltons	12	13	13	
100000 Daltons	13	13	13	13
Total	14	14	14	
Total	15	15	14	14

Table A.91 Colour measurement of blank for removed fraction mini-study on Western Pulp effluent

Sample		Absorbance		Average
Blank			0	
Std. 50	0.015	0.014	0.015	
Std. 100	0.037	0.036	0.037	
Std. 300	0.099	0.100	0.100	
Std. 500	0.167	0.169	0.168	
500 Daltons	0.007	0.008	0.008	
500 Daltons	0.007	0.008	0.008	0.008
3000 Daltons	0.041	0.041	0.041	
3000 Daltons	0.036	0.036	0.036	0.039
10000 Daltons	0.042	0.042	0.042	
10000 Daltons	0.041	0.040	0.041	0.041
100000 Daltons	0.071	0.070	0.071	
100000 Daltons	0.070	0.071	0.071	0.071
Total	0.082	0.081	0.082	
Total	0.081	0.080	0.081	0.081

Table A.92 COD measurement of blank for removed fraction mini-study on Western Pulp effluent

Sample		Peak height		Average
3000 Daltons	28	29	29	
3000 Daltons	28	28	29	29
10000 Daltons	28	28	29	
10000 Daltons	29	28	29	29
100000 Daltons	32	34	32	
100000 Daltons	35	33	35	34
Total	30	29	31	
Total	33	34	34	32

APPENDIX 12. TEMPERATURE MINI-STUDY

Table A.93 Colour measurement equilibrium isotherms with NaN_3 addition at 35°C on Western Pulp effluent

Sample	Absorbance			Average
Blank	0		0	
Std. 50	0.015	0.014	0.015	
Std. 100	0.037	0.039	0.038	
Std. 300	0.102	0.103	0.103	
Std. 500	0.173	0.173	0.173	
t=0	0.118	0.118	0.118	
t=0	0.117	0.118	0.118	0.118
t=1 h	0.098	0.097	0.098	
t=1 h	0.098	0.097	0.098	0.098
t=8 h	0.089	0.088	0.089	
t=8 h	0.088	0.088	0.088	0.088
t=20hr	0.079	0.080	0.080	
t=20hr	0.081	0.081	0.081	0.080
t=24 h	0.071	0.072	0.072	
t=24 h	0.072	0.071	0.072	0.072
t=32 h	0.063	0.063	0.063	
t=32 h	0.061	0.060	0.061	0.062
t=40 h	0.061	0.060	0.061	
t=40 h	0.061	0.059	0.060	0.060
t=48 h	0.057	0.059	0.058	
t=48 h	0.057	0.057	0.057	0.058
Blank t=48 h	0.119	0.119	0.119	
Blank t=48 h	0.125	0.125	0.125	0.122

Table A.94 COD measurement equilibrium isotherms with NaN_3 addition at 35°C on Western Pulp effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 100	0.038	0.038	0.038	
Std. 300	0.117	0.116	0.117	
Std. 500	0.199	0.200	0.200	
Std. 800	0.316	0.315	0.316	
t=0	0.130	0.132	0.131	0.131
t=1 h	0.128	0.128	0.128	
t=1 h	0.127	0.128	0.128	0.128
t=8 h	0.133	0.132	0.133	
t=8 h	0.140	0.140	0.140	0.136
t=20hr	0.133	0.133	0.133	
t=20hr	0.132	0.132	0.132	0.133
t=24 h	0.110	0.111	0.111	
t=24 h	0.108	0.108	0.108	0.109
t=32 h	0.092	0.092	0.092	
t=32 h	0.094	0.094	0.094	0.093
t=40 h	0.124	0.123	0.124	
t=40 h	0.116	0.115	0.116	0.12
t=48 h	0.113	0.113	0.113	
t=48 h	0.116	0.114	0.115	0.114
Blank t=48 h	0.120	0.121	0.121	
Blank t=48 h	0.119	0.118	0.119	0.120

Table A.95 DOC measurement equilibrium isotherms with NaN_3 addition at 35°C on Western Pulp effluent

Standard	Height of peak (mm)				Value	Average
Blank	3	3	2		3	
Std. 50	11	10	10		10	
Std. 500	106	105	100	171	121	
t=0	39	37	39		38	
t=0	41	43	42		42	40
t=1 h	42	46	46		45	
t=1 h	41	39	39		40	42
t=8 h	50	52	51		51	
t=8 h	51	50	51	50	51	51
t=20hr	50	53	53	55	53	
t=20hr	52	51	49		51	52
t=24 h	43	44	44		44	
t=24 h	42	43	42		42	43
t=32 h	32	35	33	33	33	
t=32 h	33	34	34		34	33
t=40 h	36	35	35		35	
t=40 h	35	34	36		35	35
t=48 h	36	35	35		35	
t=48 h	34	33	35		34	35
Blank t=48 h	43	43	42		43	
Blank t=48 h	40	39	42		40	42

Table A.96 pH measurement equilibrium isotherms with NaN_3 addition at 35°C on Western Pulp effluent

Sample	pH
t=0	7.79
t=1 h	8.77
t=8 h	8.92
t=20 h	9.02
t=24 h	8.82
t=32 h	8.47
t=40 h	8.42
t=48 h	8.47
Blank t=48 h	9.17

APPENDIX 13. PRACTICAL APPLICATION STUDY

Table A.97 Colour measurement of batch activated sludge testing on Howe Sound effluent

Sample	Absorbance		Value	Average
Blank	0		0	
Std. 50	0.015	0.015	0.015	
Std. 100	0.038	0.040	0.039	
Std. 300	0.105	0.104	0.105	
Std. 500	0.178	0.178	0.178	
Blank 1, t=32 h	0.076	0.076	0.076	
Blank 1, t=32 h	0.071	0.073	0.072	0.074
d=0.8 g, t=32 h	0.042	0.042	0.042	
d=0.8 g, t=32 h	0.041	0.044	0.043	0.042
d=4 g, t=32 h	0.032	0.031	0.032	
d=4 g, t=32 h	0.032	0.029	0.031	0.031
d=8 g, t=32 h	0.025	0.027	0.026	
d=8 g, t=32 h	0.022	0.023	0.023	0.024
Blank 2, t=32 h	0.069	0.067	0.068	
Blank 2, t=32 h	0.069	0.068	0.069	0.068

Table A.98 pH measurement of batch activated sludge testing on Howe Sound effluent

Sample	pH
Blank 1, t=32 h	8.36
d=0.8 g, t=32 h	8.14
d=4 g, t=32 h	7.82
d=8 g, t=32 h	7.75
Blank 2, t=32 h	8.53

Table A.99 TOC measurement of batch activated sludge testing on Howe Sound effluent

Sample	Peak height (mm)				Average
0	1	1	1		1
50	18	16	16		17
500	168	170	164	172	169
Blank 1, t=32 h	146	136	103	116	125
d=0.8 g, t=32 h	118	128	122		123
d=4 g, t=32 h	188	186	186	180	185
d=8 g, t=32 h	180	192*	192*	192*	189
Blank 2, t=32 h	100	95	90	115	100

*Maximum value

Table A.100 TSS measurement of batch activated sludge testing for Howe Sound effluent

Sample	Sample size (L)	Dish & filter (g)	After sample & drying (g)	Difference (g)	Blank adjust. (g)	TSS mg/L
Blank 1, t=32 h	0.015	1.1215	1.1218	0.0003	0.0006	40
d=0.8 g, t=32 h	0.015	1.0884	1.0884	0.0000	0.0003	20
d=4 g, t=32 h	0.015	1.0978	1.0982	0.0004	0.0007	47
d=8 g, t=32 h	0.005	1.1555	1.1563	0.0008	0.0011	220
Blank 2, t=32 h	0.015	1.1103	1.1101	-0.0002	0.0001	7
Filter blank		1.1074	1.1071	-0.0003	0.0000	

Table A.101 BOD measurement of batch activated sludge testing for Howe Sound effluent

Sample	Volume of sample (mL)	Bottle number	Initial DO (mg/L)	Final DO (mg/L)	Value (mg/L)	With dilution water adjustment
Blank 1, t=32 h	7.0	44	9.74	5.96	32	31
	2.0	67	9.84	7.78	62	60
d=0.8 g, t=32 h	2.0	61	9.76	5.76	120	118
d=4 g, t=32 h	0.5	64	9.81	3.72	731	729
d=8 g, t=32 h	0.5	69	9.78	0.36	1130	1129*
Blank 2, t=32 h	7.0	50	9.78	6.57	28	26
	2.0	59	9.85	7.96	57	55

* Not a valid measurement