Biodegradability Enhancement in Municipal Wastewater Using New Environmentally Friendly Biostreme

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

MASTER OF APPLIED SCIENCE

Parin Izadi

Graduate Program in

Civil Engineering

York University

Toronto, Ontario

August 2017

Abstract

Microorganism's metabolic rate is the ultimate operational basis of diverse biological treatment processes. A variety of assorted factors can affect the biological wastewater treatment's metabolic rate. One of the prominent means, is the use of supplements such as trace metals and vitamins. Both batch and continuous experiments have been used to study the effect of a variety of trace metals solution (named biostreme solution) and vitamins solution on biological organic removal process.

The results show that, in average addition of 500 ppm of the biostreme had the highest rate of organic removal. The colloidal removal was enhanced by a rate of between 65 to 75% in specific batch tests. When the wastewater was supplemented with concentrations of biostreme individually and in a mixture with the vitamins solution, chemical oxygen demand fractionation showed a significant decrease in the colloids, however there is a different effect observed when returned activated sludge was used as the seed.

Acknowledgement

I would like to gratefully acknowledge my astonishing companions in this journey. First of all, I owe an enormous depth of gratitude to my supervisor; Professor Ahmed Eldyasti, whose support, patience, knowledge, care and wisdom helped me become a better researcher and engineer.

I would like to send my sincere regards to my kind and amazing colleagues, Moomen soliman, Ahmed Fergala, Ahmed AlSayed, Mahmoud Mansour, Basma Sobhi and Bita Pihani for their encouragement and support in the hard times of lab works.

My incredible friends, Ardavan, Negar and Amir, were the reason for all the great and cheerful times in this journey. I would like to thank my wonderful aunt and her beautiful family, Hadis, Amir and Nikki, you are my family away from home.

Lastly and most of all, I would like to thank my beloved family; my exceptional parents; you have always been my greatest role models and my source of power. Thank you for your endless love and support.

My precious twin sister, Parnian, you are the reason why my life is so great and stunning. I thank god, every day for having you in my life.

I would like to dedicate my thesis to an extraordinary soul, an exceptional person and a loving spirit; *my grandfather*.

Table of Contents

List of tables:

List of figures:

Acronyms

AS: Activated sludge from lab system

ATP: Adenosine triphosphate

BOD: Biochemical oxygen demand

BOR: Biological organic removal

COD: Chemical oxygen demand

DO: Dissolved oxygen

EBPR: Enhanced biological phosphorus removal

F/M ratio: Food to microorganism's ratio

MBR: Membrane bioreactor

NIT: nitrification

PAO: Polyphosphate accumulating organisms

PHA: Phytohemagglutinins

RAS: Returned activated sludge

TSS: Total suspended solids

TOC: Total organic carbon

UASB: Up-flow anaerobic sludge blanket

VSS: Volatile suspended solids

WW: Wastewater

Chapter 1: Background

Pollution and waterborne disease are two of many various results of direct discharge of wastewater into environment. Biological treatment of wastewater was developed in early twentieth century; and now, biological pathways are the basis of wastewater treatments around the world in the recent years. Biological processes are based on a simple concept of removing pollutants using living microorganisms. The microorganisms (bacteria, protozoa, microbes, etc.) maintain in the waste streams by using the organic matters for growth and synthesis. The treated water (effluent) can be discharged to receiving waters.

However, there is a simple concept behind biological processes, the control and monitoring of the process is extremely complicated. There is an extensive amount of variables and factors affecting the process performance. A few of many diverse factors are, composition of bacteria fluctuations', influent's flowrates, chemical compositions, pH, temperature, toxicants etc.

Municipal wastewater mainly consists of organic carbon (soluble matters and particulates). Approximately two third of the waste stream is made up of particulate matter in which half can settle. Particles with a size range of 1nm to 100μm are referred to as colloids which require extra processes to be removed.

Organic matter's segment of the wastewater consists of readily biodegradables which comprise of; proteins, amino acids, fatty acids, carbohydrates and fats. The average C: N: P ratio in municipal wastewater is roughly 100: 17: 5 or 100: 19: 6 which is just about ideal, intended for bacterial growth.

The dominant organisms (wide range of approximately 300 species) used for biological wastewater treatment processes are bacteria. Bacteria are one of the smallest yet most populated species amongst all. There microorganisms consist of a cell with a size between 0.5 to 2 µm. There is a membrane boundary on the outer layer of the structure which regulates the in and outflow of ions and molecules from the environment. The cell membrane is protected by a rigid cell wall made of sugar polymers. The inside sector of the bacterial cell includes cytoplasm, reaction chemicals and enzymes.

Organic removal from mixed liquor is completed through ingestion by bacteria. The metabolism of the ingested carbon compounds takes place inside of the bacteria where numerous chemical reactions happen simultaneously. The overall pathway of the reactions is conversion of substrate to products, in addition, there are chain reactions in the mixed liquor in which each individual reaction occurs in presence of a specific enzyme. The major divisions of metabolism are catabolism and anabolism.

Bacterial growth is one of the vital factors in biological wastewater treatment processes. There are diverse conditions required for bacterial growth, for instance some of the basic principles are the substrate concentration, nutrient availability, oxygen concentration, temperature and toxicity. However, the main substrate requirement in the process is carbon but the process is also dependent on different macro and micronutrients. Nutrients are required for maximum bacterial growth and optimized treatment. Lack of critical nutrients may result in incomplete treatment due to low capability of optimal growth.

Macronutrients can be divided in to six core elements; carbon, oxygen, hydrogen, nitrogen, phosphorus and sulphur, in which the primary nutrients are carbon, nitrogen and phosphorus. Requirement for trace elements is strongly reliant on the bacterial cell composition. However, trace nutrients are essential for the biological processes, but extra amount of these additives can lead to adsorption in cell walls. The vital concentration is based on the organic sludge system, rate of bacterial growth, source of the wastewater and the cell retention time.

In specific circumstances where there is restricted amount of nutrients available to the microorganisms, external supplementation can result in organic degradation improvement. Sufficient concentrations of both trace metals and vitamins are vital for all diverse genera present in the activated sludge system. Limited concentrations of specific nutrients can result in unbalanced systems, low quality effluents, poor efficiency and problems in sludge handling. A common example of micronutrients deficiency consequence is; poor flocculation, pin point flocs and dispersed growth. Nutritional balance has the potential to improve biological treatment of wastewater.

Essential metals commonly have a vital role as cofactors for enzymes. Some of the important microelements required by microorganisms includes; sodium, potassium, iron, magnesium, calcium, cobalt, manganese, molybdenum and etc. (Wang, 2012)

Some of the general functions can be summarized as following; sodium is compulsory for cell maintenance and growth, iron has a critical role in cell respiration, potassium is required by number of enzymes while magnesium can act as a stabilizer for “ribosomes, cell membranes and nucleic acids”. molybdenum is required to aid conversion of nitrogen to ammonia, manganese is used as catalyst for phosphate transfer and cobalt is one of vitamin B_{12}’s key components. (Wang, 2012)

There is a probability that a microorganism has a substantial ability to synthesize with presence of a limited amount of microelements, but usually there are broader requirements, regarding bacterial growth. One of the main growth factors are vitamins. Vitamins can be defined as organic compounds which function as enzyme cofactors. The vitamins desired by microorganisms are often, Thiamine (B_1), Biotin, Pyridoxine (B_6) and vitamin B_{12}. (Wang, 2012)

Biotin can function as a fixator of carbon dioxide as well as carrier of carboxyl in the reactions. Riboflavin and thiamin can perform dehydrogenation and decarboxylation, respectively. (Wang, 2012). Metals and vitamins both can have either a beneficial or a detrimental effect on biological processes, depending on the process type, the supplement type and the concentration in which the micronutrient is added.

1.2. Problem statement

Alterations in wastewater treatment legislations, shifted the prominence towards biological processes. The operational principle of the biological treatments is grounded on the metabolic rate of the altered microorganisms at different environmental conformations. The rate and potential of decomposition rests on the microbial activities and, slow and partial degradation can be concomitant to several different factors such as specific micronutrients limitations.

Explicit nutrients are required for the metabolic activity; furthermore, adequate micronutrients are critical to maintain all the multiplicity of diverse species present in the wastewater stream.

Poor effluent quality, bulking and foaming due to insufficient solids removal as well as low metabolic rates have been accredited to low micronutrient concentrations in the biological process.

Maximizing the diversified population in the activated sludge using the full co-metabolism, high rate of colloids removal and high metabolic rate can be reached by attaining the optimum amount of micronutrients (trace metals and vitamins) for each specific biological process. Micronutrients have the potential to stimulate the biological wastewater treatment process by improving the bacterial community diversion and performance. In the circumstances, which treatment plants are handling a nutrient-limited wastewater stream influent, macro and micronutrients supplementation can result in a boosted degradation of organic matters.

1.3. Objectives

With the aim of concluding the effect of the biostreme (a solution which comprised of trace concentration of metals dissolved in water) and vitamins solution on the activated sludge process, a series of diversified biodegradability batch tests were conducted.

The specific objectives of this research are:

- Achieving stable and complete activated sludge process (Biological organic removal, BOR), using municipal wastewater as the influent.
- Studying the effects of additives (trace metals and vitamins) referred to as biostreme and vitamin solution on continuous BOR process.
- Analyzing the effects of the biostreme and vitamins on several biodegradability tests regarding the biological organic removal rate and colloidal and particulates removals.
- Study both the influence of different seeds and various concentrations of the supplements on the batch biodegradability tests.
- Understanding the removal rates alteration due to different organic loading rates and supplementation.

1.4. Thesis layout

This thesis includes six main chapters which focus on the background, literature review, the methodology and the results and conclusions respectively.

In the first chapter a brief background on biological wastewater treatment is stated.

In the second chapter, literature review, a comprehensive study on five main biological wastewater treatment processes; BOR, nitrification, denitrification, EBPR and AD was done. The effects of different additives as well as the fundamental reasons for each supplementation is mentioned in details.

The four remaining chapters comprise of the material and methods of the laboratory experiments. The chapters also include all the diverse steps taken towards the main objective of the research. In each chapter the results of the several experimental phases, measurements and analysis are studied thoroughly.

1.5. Contribution of the thesis

With the intention of enhancing biological wastewater and sludge treatment processes in terms of both biological organic and solids removal rates, this project focuses on developing new optimum micronutrient supplement configuration as well as its implementation in to the design of the biological processes.

The new environmentally friendly biostreme offers a balanced and easily accessible micronutrient package that leads to vastly bioavailable biochemical for microorganisms' existent in the waste stream. Utilization of this additives offers more alternatives for systems with low rates of organic removal as well as systems struggling with high percentage of colloidal matters present in their effluents.

biostreme and vitamin's solution were added to biological organic removal process in combination and individually to assess the effects of external supplementation, trace metals and vitamins different concentrations and additives interactions on the organic and solids removal rates.

With the intention of implementing this idea, several biodegradability tests using three different biomass sources (two returned activated sludge and yeast) and municipal wastewater as the sample were performed.

Chapter 2: Literature Review

2.1. Introduction

Due to economic and potency factors, there is a great amount of attention paid to biological processes. There are a wide range of biological wastewater treatment processes used worldwide. Biological treatments are basically eliminating pollutants present in water, by adapting living microorganisms. Biological processes are characterized based on the environment, the biological transformations, design and microorganisms involved. The most significant processes amongst all, are as following; biological organic removal, nitrification, denitrification, enhanced biological phosphorus removal and anaerobic digestion.

Success of biological processes is determined by a proper, dynamic and diversified population of microorganisms in the system, moreover the organic waste is consumed by the microbial community as food source. This source is used for new cell synthesis (energy) and cell maintenance (degradation), which requires micronutrients for proper energy endowment and biodegradation.

Most wastewater sources include biodegradable contents. The key objective of biological processes is to decline the organic and inorganic compounds existing, associated with nutrients (phosphorus and nitrogen) removal (PETER, 2008).

The metabolic rate is controlled by the microbial activity; in addition, it can designate the degradation rate of the microorganisms. A variation of different factors can have vital effects on the efficiency and metabolic rate of these processes. Bacterial growth increases tremendously in favorable conditions and environments (PETER, 2008).

In biological processes, microorganisms are predominantly in charge of organic compound's degradation. The fundamental working basis of biological treatments is the microorganism's metabolic rate. With the intention of realizing the crucial significance of micronutrients for biological processes a comprehensive understanding of the microbial growth, metabolism and dynamics is essential(Burgess, Quarmby, & Stephenson, 1999a).

Biodegradation is fragmentation of materials by living organisms such as bacteria, fungi etc. Biodegradables are mostly organic materials that play a role as nutrients for the microorganisms.

Some crude factors such as wastewater's chemical oxygen demand (COD), biochemical oxygen demand (BOD), volatile and total suspended solids have been conventionally used to characterize the organic matters present in the wastewater. The overall concentration of the organic matter is measured by COD and volatile suspended solids (VSS), while BOD measurements result in total biodegradable portion of the organics.

The concentration of biodegradable organics in wastewater samples is assessed through BOD tests, such as organic matter removal performance evaluation in wastewater treatment plants.

In the BOD tests the bacterial consumption of oxygen is measured during a specific incubation time. The results show the biochemical organic matters degradation (cBOD), although there is a possibility that it also includes the oxidation of inorganic materials such as sulfides and ferrous iron. In cases where there are no nitrification inhibitors present, the test measures the nitrogenous biochemical oxygen demand as well as the carbonaceous part. Total BOD consists of nBOD and cBOD.

The conventional methods do not show the detailed information on the biodegradable fractionation and composition. In the past years, wastewater treatment was restricted to organic carbon removal.

Increasing pollutions, resulted in stringent limitations, where the new approach to wastewater treatment processes influenced the wastewater characterization concept. Optimization of the nutrient removal requires a comprehensive understanding of the wastewater characteristics with a focus on COD fractionation (Orhon, Ateş, Sözen, & Çokgör, 1997).

COD fractionation is the pathway to achieving detailed characterization of wastewater. There are three main fractionations for COD, first the active agents which consist of the heterotrophic microbial biomass, second the substrate for the biomass which can be a mixture of both readily and slowly biodegradable materials, the third part of the organic matter is the non-biodegradable which is referred to as inert. Size distribution of the existing pollutants in the wastewater meant for characterization is considerably important (Vollertsen & H vitved-Jacobsen, n.d.).

It has been observed for treated and untreated wastewater, particles with sizes over 1 micro meter constitute a considerable portion of the overall organic matters. Different studies show that the raw influent municipal wastewater contains between 12-64% solubles (<0.08 micrometer), 7-15% colloids (0.08-1 micrometer), 12-38% supra-colloidal matters (1-10 micrometer) and 12-45% settleables (>100 micrometer).

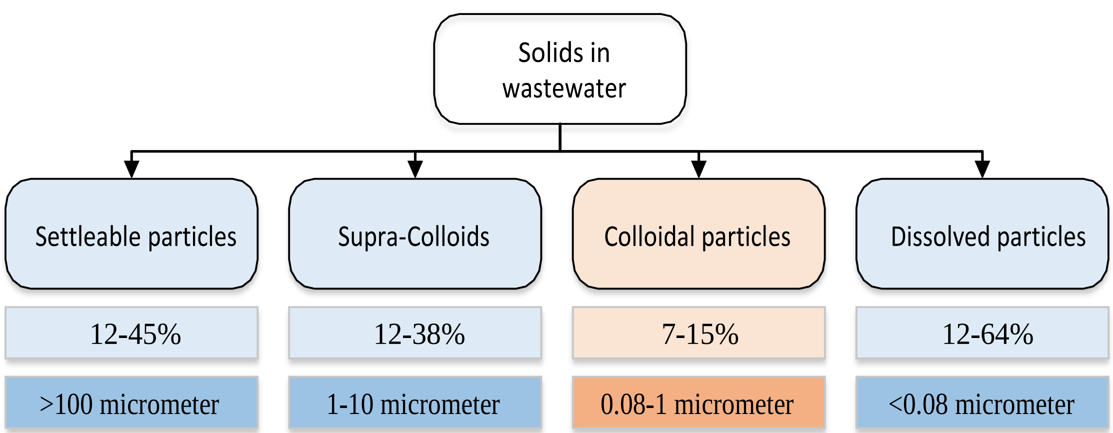


Figure 2- 1: Wastewater solids categorization based on size

Complete removal of all sorts of particles from the wastewater is crucial for the effluent reuse applications. Membrane filtration is a developing system in wastewater treatment pathways. A significant limiting factor for these membranes is directly related to the solids characteristics of the input while fouling is mainly caused by supra-colloids and small colloids (Orhon et al., 1997).

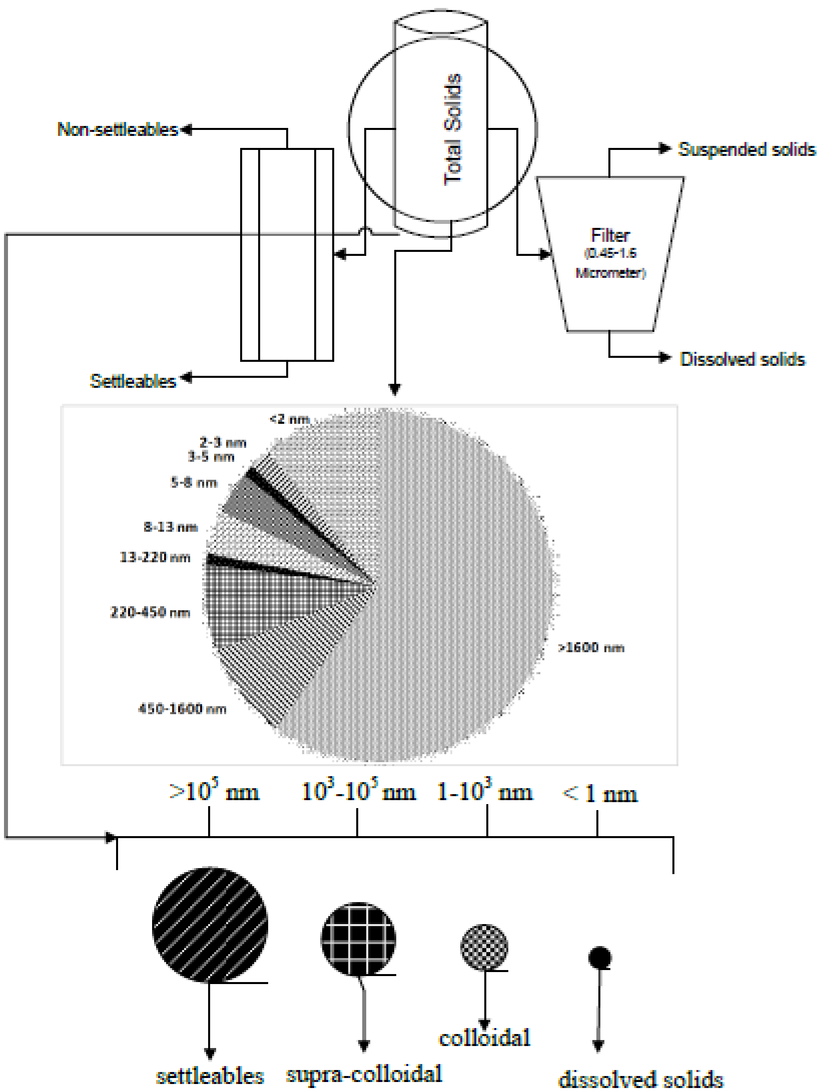


Figure 2- 2: Wastewater's solids groups and sizes

A variety of assorted factors can affect the biological wastewater treatment metabolic rate, biodegradation, size distribution and solids removals. One of the prominent means for increasing the metabolism of the bacteria involved in the removal process, is the use of supplements such as trace metals and vitamins.

In order to enrich the biological wastewater treatment a variety of different factors must be considered. Parameters that effect the effectiveness can vary from temperature and pressure to the nutrients supplements. Low degradation rates may be a result of micronutrients limitation, in addition, one of the influential ways to increase the rate, is the use of supplements such as microelements and trace metals.

Other than macronutrients such as C, H, N, O, S and P, which are essential for biological cells, availability of sufficient amounts of micronutrients can have a considerable effect on the metabolic rate. These elements support the growth of microorganisms and can be divided into two main categories; Metals (ions) and vitamins.

Generally, a convoluted mixture of nutrients is necessary in biological treatment processes. This mixture often includes both macronutrients and micronutrients. Absence of some explicit nutrients may result in unbalanced Bio-systems as well as reduction in microbial growth and treatment efficiency and issues in handling the process (Li, Lei, Zhang, & Sugiura, 2006).

However, presence of micronutrients can have a compelling positive effect on biological processes, optimum concentration estimation is extremely crucial. Exceeding the limit may result in adsorption of trace elements in the cell walls (Burgess et al., 1999a).

Essential metals generally have a vital role as cofactors for enzymes. Some of the essential microelements required by microorganisms include; sodium, potassium, iron, magnesium, calcium, cobalt, manganese, molybdenum etc. (Wang, 2012).

Some of the general functions can be summarized as following; sodium is needed by the cell for maintenance and growth. Iron has a critical part in respiration in the cell. potassium is required by many enzymes and magnesium can act as a stabilizer for" ribosomes, cell membranes and nucleic acids". molybdenum is required to aid conversion of nitrogen to ammonia. manganese is used as catalyst for phosphate transfer and cobalt is one of vitamin B_{12}'s components (Wang, 2012). Specific microorganisms have a significant ability of synthesizing a few of the microelements, but generally there are broader requirements, regarding the growth. One of the main growth factors are vitamins, which can be defined as organic compounds that function as enzyme cofactors. The vitamins desired by microorganisms are often, thiamine (B_1), biotin, pyridoxine (B_6) and vitamin B_{12} (Wang, 2012).

Biotin can function as a fixator of carbon dioxide as well as transfer of carboxyl in the reactions. Riboflavin and thiamin can perform dehydrogenation and decarboxylation, respectively (Wang, 2012).

Metals and vitamins both can have either a beneficial or a detrimental effect on biological processes, depending on the process type, the supplement nature and the concentration in which the micronutrient is added.

Basically, biological treatment of wastewater can be studied in two main categories, of suspended growth and attached growth systems. Suspended processes which can also be assigned as activated sludge process, are different from attached systems in which the microorganisms attach to a surface media. The attachment can result in an inert support (Wang, 2012).

In suspended growth, by providing suitable mixing methods the biomass containing the microorganisms is in liquid suspension. Mostly suspended processes are used for aerobic systems but there are anaerobic suspended growth systems for high organic concentrations, while in an attached system, there is an “inert packing material”. The inert packing attached growth is known as the biofilm while, both aerobic and anaerobic processes can be performed as attached systems. The suspended systems are more sensitive to toxicants and have less effective mass transfer due to the complete mixing. On the other hand, the attached systems have a significant mass transfer. The possibility of wash outs is higher in suspended systems. The system’s efficiency in suspended growth is directly connected to secondary clarifier while in attached systems the efficiency is independent of the clarifier (Wang, 2012).

2.2. Biological organic removal

In the conventional BOR processes the ordinary heterotrophic organisms (OHO’s) present in the process, remove the organic matters with the intention of producing additional biomass by using oxygen as their source for respiration.

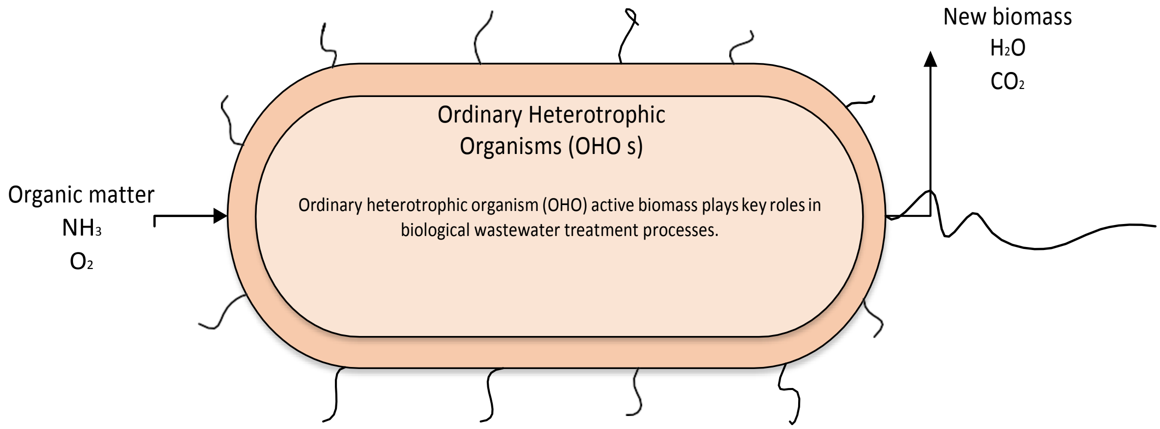


Figure 2- 3: Heterotrophic bacteria definition

Breakdown of some of the organic matters, results in the required energy for growth and maintenance of heterotrophic microorganisms.

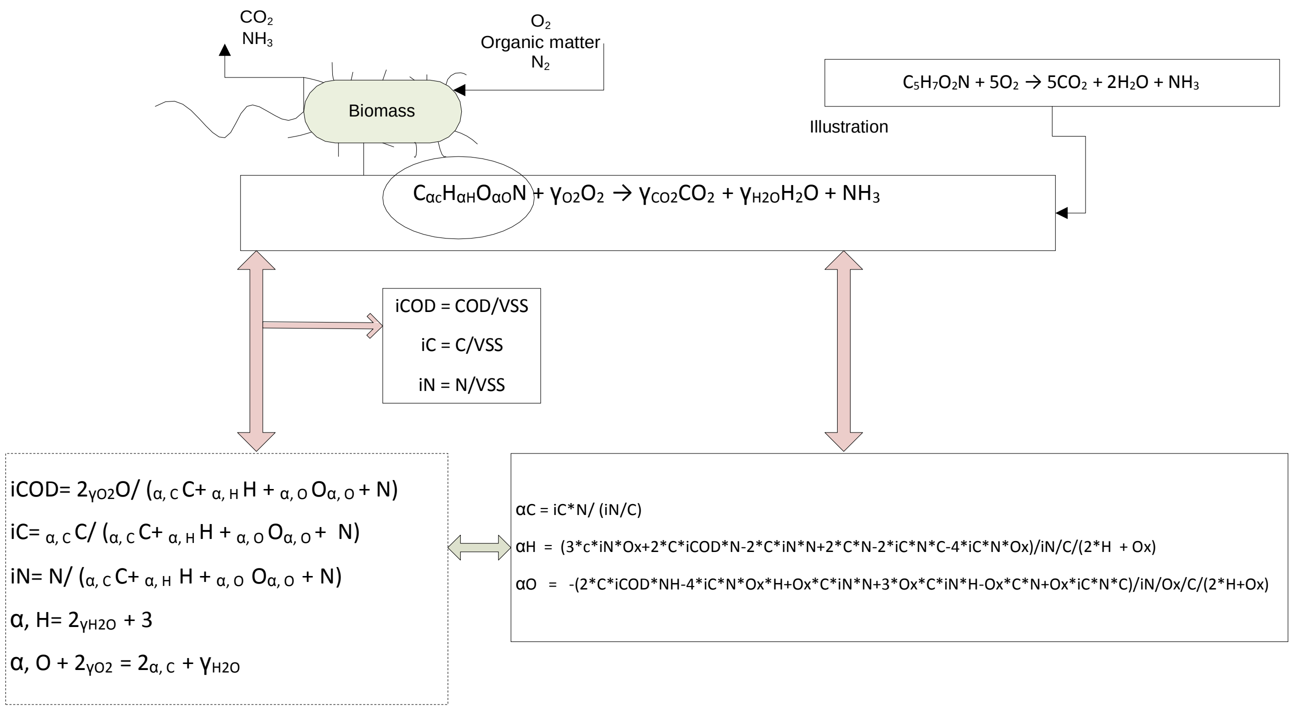


Figure 2- 4: BOR process stoichiometry

Some environmental factors which can affect the biological growth, include temperature, mixing regime, toxic measures and pH. Organisms' growth has different stages based on time. The initial phase is a logarithmically growth which is followed by a declining growth phase in which there is a restricted nutrient source, the microorganisms reach a maximum concentration subsequently, and from then on, the decay phase begins as the cells start to die. Removal process comprises of two core parts; catabolic and anabolic process. In the catabolic process, electron transfer in oxidation-reduction reactions, generates the required energy for cell synthesis, while the anabolic process necessitates energy for new cell synthesis, as well as carbon from the organic matters, for cell growth. (Konopka, Oliver, & Jr., 1998)

The exact stoichiometry for BOR process is not straight forward as there are different carbon sources that can be involved, nonetheless as an illustration for this process, the following equations can be used:

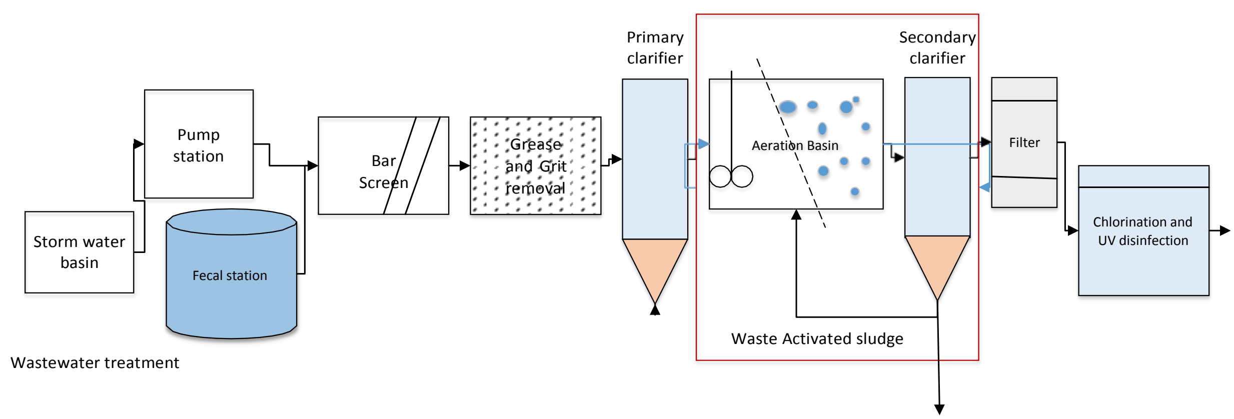


Figure 2- 5: Activated sludge system process scheme

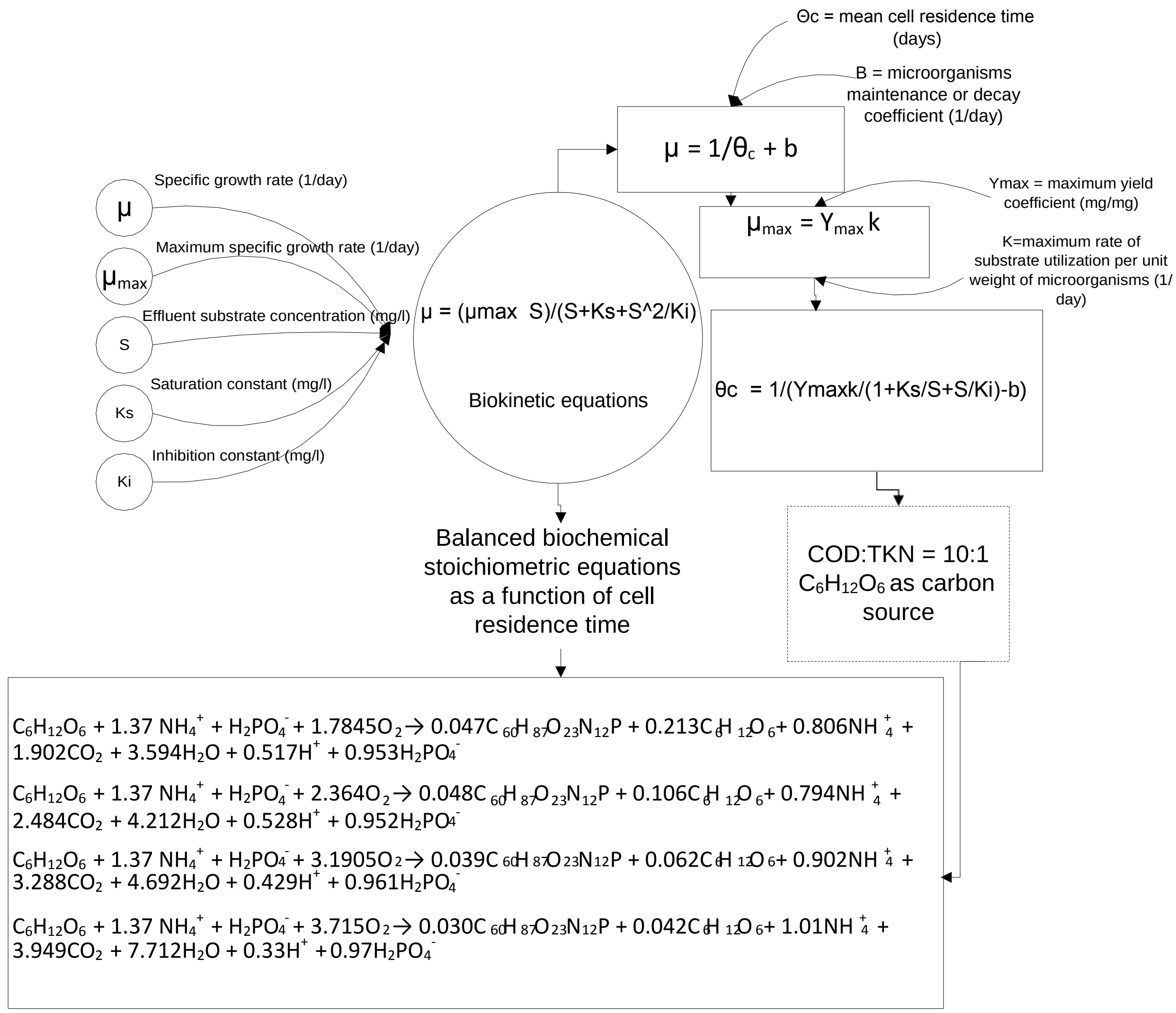


Figure 2- 6: BOR bio-kinetics and stoichiometry

There are a wide variety of industrial wastewaters that are resistant to the biological based processes, while these processes may be effective for degradation of domestic wastewaters. By providing sufficient amount of micronutrients and balancing the process, a noticeable number of issues associated with the treatment can be omitted (Burgess et al., 1999a).

In biological organic removal, the cells require six basic macro nutrients for their metabolic processes. These macro nutrients are carbon, oxygen, hydrogen, sulphur, nitrogen and phosphorus (Jefferson, Burgess, Pichon, Harkness, & Judd, 2001a).

Metals such as Ca, K, Fe, Mn, Zn, Mo and Na have a significantly beneficial effect while Mg, Cu, Al and Co result in enhancement of the process if they are used in a specific range of concentrations. Exceeding the range in the second group causes a detrimental effect on the metabolic rate and may result in a total inhibition of organic removal. Vitamins, for instance niacin, thiamine, lacto flavin and pantothenic acid have a positive effect on organic removal process. Other advantageous vitamins are biotin, pyridoxine, B_1, B_2, B_6 and B_{12} in diverse concentration ranges (Burgess, Quarmby, & Stephenson, 1999d).

Ca addition improves the flocculation while it can also affect the membrane permeability. Addition of Ca and Mn improves the COD removal probably because of permeability increase as a result of Ca supplementation which improves, Mn access to the bacterial cells and results in speeded up metabolism. Combination of Ca with other micronutrients results in reduced COD removal and improved respiration. Ca and Zn independently improve the treatment, even though they turn out to be inhibitory in combination, this is when, Ca can enhance the effects of niacin and Mn (Burgess, Quarmby, & Stephenson, 1999b), (Burgess, Quarmby, & Stephenson, 1999c), (Jefferson et al., 2001a), (Burgess, Quarmby, & Stephenson, 2000).

Mo is generally a restricted nutrient which is one of the two single additives with stimulating respiration effect. Fe is a growth factor for bacteria, fungi and algae, in addition it aids the transport of electrons in cytochromes. This trace metal has a role in synthesis of catalase peroxidase and aconitase. Ion reduction for floc formation is also another effect of Fe. Mg is considered to be an enzyme activator for heterotrophic bacteria (Burgess et al., 1999b), (Burgess et al., 1999c), (Jefferson et al., 2001a), (Burgess, Quarmby, et al., 2000).

Mn is the other single additive with stimulating respiration effect which is also an enzyme activator and influences respiration and process performance. K influences both the transport system and osmotic balance in the bacteria (Burgess et al., 1999b), (Burgess et al., 1999c), (Jefferson et al., 2001a). Cu is an enzyme activator but it is required in small quantities and it can easily inhibit the process, the beneficial effect of this metal is its ability to chalet other substances and reduce their toxicity (Burgess et al., 1999b), (Jefferson et al., 2001a), (Clark & Stephenson, 1998a).

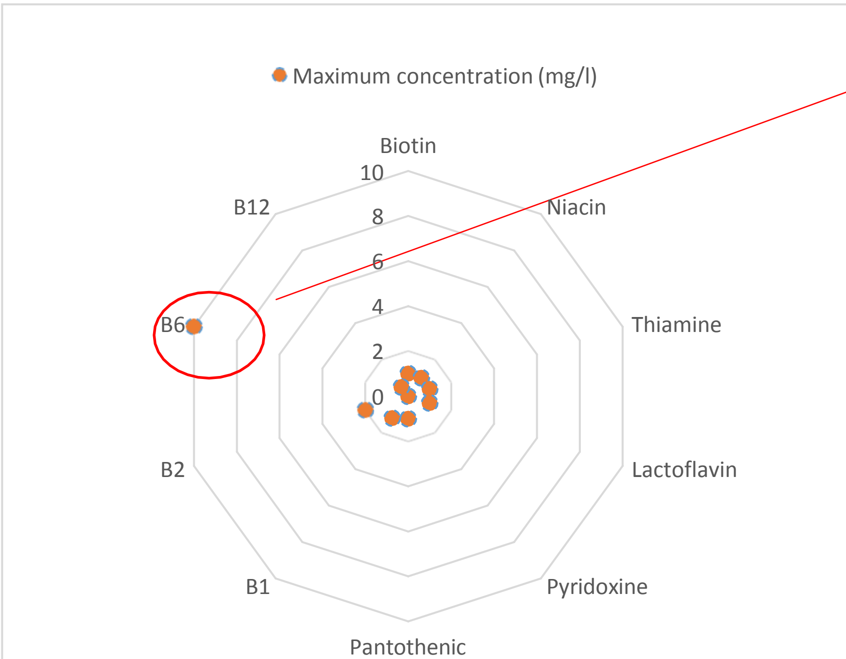
Co is also a metallic enzyme activator and dissociable on active site of enzymes, on the other hand this metal can also activate carboxypeptidase for synthesis of vitamin $B_{12,}$ nonetheless it might also become toxic and inhibitory to the process. Co can cause a 50% increase in B_{12} synthesis in dosage of 1 mg/l which is a stimulating effect, however there are no observed improvements in the COD and BOD removals. This micronutrient is an enzyme activator and has an effect on respiration and process performance (Burgess et al., 1999b), (Burgess et al., 1999c), (Jefferson et al., 2001a), (Clark & Stephenson, 1998a).

Zn principally stimulates the cell growth; on the other hand, it also causes an inhibitory effect at 1 mg/l. Zn concentration do not enter the bacterial cell but it contests for extracellular binding sites. It stops adsorption and metabolism of organics, while it can also interact with other metals and aggravate their inhibitory influence and reduce the biodegradation rates. Zn is an enzyme activator and influences respiration and process performance. Ni is a stimulant for certain enzymes, and it is required for maintenance of the biomass, nevertheless it can cause metabolism inhibition in specific cases (Burgess et al., 1999b), (Burgess et al., 1999c), (Jefferson et al., 2001a).

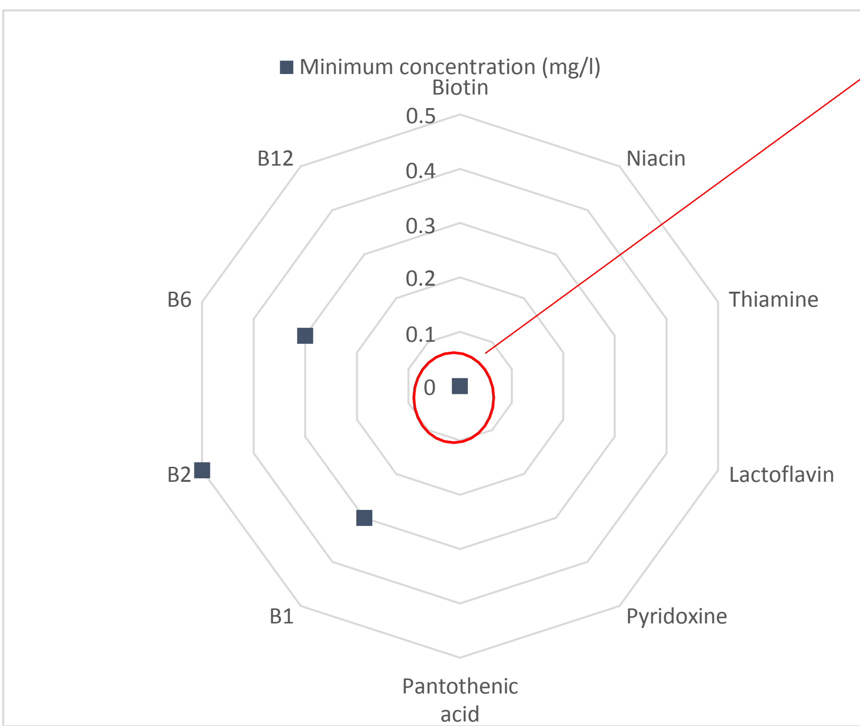
Figure 2- 7: Effects of addition of trace metals on BOR process

Thiamin is the most effective vitamin in activated sludge however, since certain bacteria can generate this vitamin there is a possibility that there are adequate dosages of it in wastewater

(Burgess et al., 1999b). Lactoflavin is considered to be vital for the cell metabolism but since autotrophs are restricted in quantity in activated sludge, it might not be necessary to add lactoflavin to the sludge (Burgess et al., 1999b), (Burgess et al., 1999c), (Burgess, Quarmby, et al., 2000).



High maximum concentration for B_6 compared to other vitamins



Most vitamins have minimum concentrations around 0 mg/l

Figure 2- 8: Effects of addition of vitamins on BOR process

The greatest improvement is when 1.0 mg/l of niacin is added to the activated sludge which results in increase in COD removal. In some of the studies it is stated that there isn't any special requirement for niacin supplementation, as most of the present bacteria can produce sufficient amounts of it in the sludge (Burgess et al., 1999b), (Burgess et al., 1999c), (Burgess, Quarmby, et al., 2000).

The suggested additive for the BOR process is a mixture of metals and vitamins considering both the effects and costs.

Table 2-1: Optimum composition of additives for BOR process

		Chemical properties
	Component	Concentration (%)
Metals	Fe	0.00001-0.00004
	Zn	0.00001-0.0001
	Ca	0.00004-0.00014
	Mn	0.00001-0.00005
	K	0.00008-.0003
Vitamins	Niacin	0.0001
	Lactoflavin	0.0001
	Pyridoxine	1e-9
	Vitamin B_{12}	5.0000000000000004e-8

The intention of choosing this set of metals, is that Fe and Zn are vital metals for this process, in addition, the costs for both metals are practically low. Ca, Mn, Mo and K are three stimulatory metals for BOR, even though considering the cost, Mo is omitted. Cu and Al have extremely precarious range of concentration and can result in detrimental effects with a minor deviation as of the range. Ca and Mn are a beneficial couple for BOR. Niacin, Lactoflavin and Pyridoxine are the most essential vitamins for this process moreover, Vitamin B_{12} is one of the beneficial additives for furthermost of the biological processes.

There are no conflicts concerning the concentrations stated in table 2-1 and the restrictions for wastewater metal concentrations.

Some of the fundamental reasons for the stated effects detected in the BOR process are listed below:

- **Ca:** Ca addition improves the flocculation; in addition, it can also affect the membrane permeability. Addition of Ca in combination with Mn improves the COD removal probably as a result of permeability increase by Ca and allowing Mn to access the bacterial cells better, leading to speeded up metabolism. Combination of Ca with other micronutrients results in reduction of COD removal and escalates respiration. Ca and Zn independently improve the treatment; this is when inhibitory effects were observed in combinations.

- **Mo:** Mo is mostly a limited nutrient. It is one of the two single additives with stimulating respiration effect.

- **Co:** Co can cause a 50% increase in B_{12} synthesis in dosage of 1 mg/l which is a stimulating effect but it doesn't improve the COD and BOD removals. This trace metal is an enzyme activator and influences respiration and process performance.

- **Zn:** Zn basically stimulates the cell growth but it also causes an inhibitory effect at 1 mg/l. Zn concentration doesn't enter the bacterial cell but it contests for extracellular binding sites, moreover it stops adsorption and metabolism of organics. Zn interacts with other metals and aggravates their inhibitory influence and reduces the biodegradation rates. Zn is an enzyme activator and influences respiration and process performance.

- **Mn:** Mn is the other single additive with stimulating respiration effect. Mn is enzyme activator and impacts respiration and process performance.

- **Thiamin:** Thiamin is the most imperative vitamin in activated sludge but since certain bacteria can generate this vitamin there is a possibility that there won't be requirements for supplementation.
- **Lactoflavin:** Lactoflavin is considered to be vital for the cell metabolism but since autotrophs are limited in quantity in activated sludge, it might not be necessary to add lactoflavin to the sludge.
- **Niacin:** The greatest improvement was when 1.0 mg/l of niacin was added to the activated sludge which resulted in increase in COD removal. In some of the studies it is stated that there isn't any special necessity for niacin supplementation, as most of the bacteria can produce sufficient amounts of it in the sludge. This statement can be accurate since the optimum amount for niacin is 1.0 mg/l. Industrial waste water doesn't contain adequate quantity of niacin.
- **Niacin and pyridine:** Pyridine provokes activity of niacin.
- **A, D, E and P:** Vitamins A, D, E and P are required by most of the microorganisms. Addition of these vitamins can improve the efficiency of the process and decrease the biomass generation as well as improving the energy yield.

- **Pantothenic acid:** Pantothenic acid is a growth factor for initial cell growth. This vitamin has its highest activity in presence of vitamins group B. It acts synergistically with biotin and pyridoxine. Deficiencies in this vitamin can cause reduced nitrogen and phosphorus removals.
- **Biotin:** Biotin is usually found in large quantities as most of the enter bacteria produce it. It acts synergistically with pantothenic acid and pyridoxine.
- **Vitamin B_2:** Vitamin B_2 has a role in growth. It has been observed that some of the bacteria species can produce this vitamin in special environment conditions.
- **Vitamin B_6:** Vitamin B_6 is a pyridine derivative active in many metabolic processes. It is a specific growth factor.
- **Vitamin B_{12}:** Vitamin B_{12} is required for growth. A cobalt containing complex is generated with the addition of this vitamin.
- **Vitamin K:** Vitamin K is a constituent of respiration chain (Jefferson et al., 2001a) (Burgess et al., 1999b) (Burgess et al., 1999a) (Clark & Stephenson, 1998b).

Combination of micronutrients results in interactions, which results in complications in process prediction. These interactions can be influenced by metal species and concentrations, operating conditions and influent characteristics, present microorganisms, sludge age and even the order of micronutrients addition.

Inhibitory effects can be caused by the prevention of COD degradation resulting in metabolism's suppression. High metabolic activities can be a result of altering metabolism to degrade more to obtain sufficient energy.

2.2. Nitrification

The significance of the nitrification process has been understood in recent years. Nitrification can be defined as a biological process in which ammonia is converted to nitrite and nitrate in two steps. The capability of biological nitrogen control processes relies on the ability of the organisms responsible for oxidation of ammonia to nitrate (A. C. Anthonisen, R. C. Loehr, T. B. S. Prakasam, 1976).

One of the cost effective methods to remove the excessive ammonia from water streams is biological nitrification process, where the ammonia containing wastewater is treated under aerobic conditions with bacteria in aerobic tanks through two separate steps: ammonia is oxidized to nitrite, and then nitrite is oxidized to nitrate via *Nitrosomonas* and *Nitrobacter*, respectively (A. C. Anthonisen, R. C. Loehr, T. B. S. Prakasam, 1976).

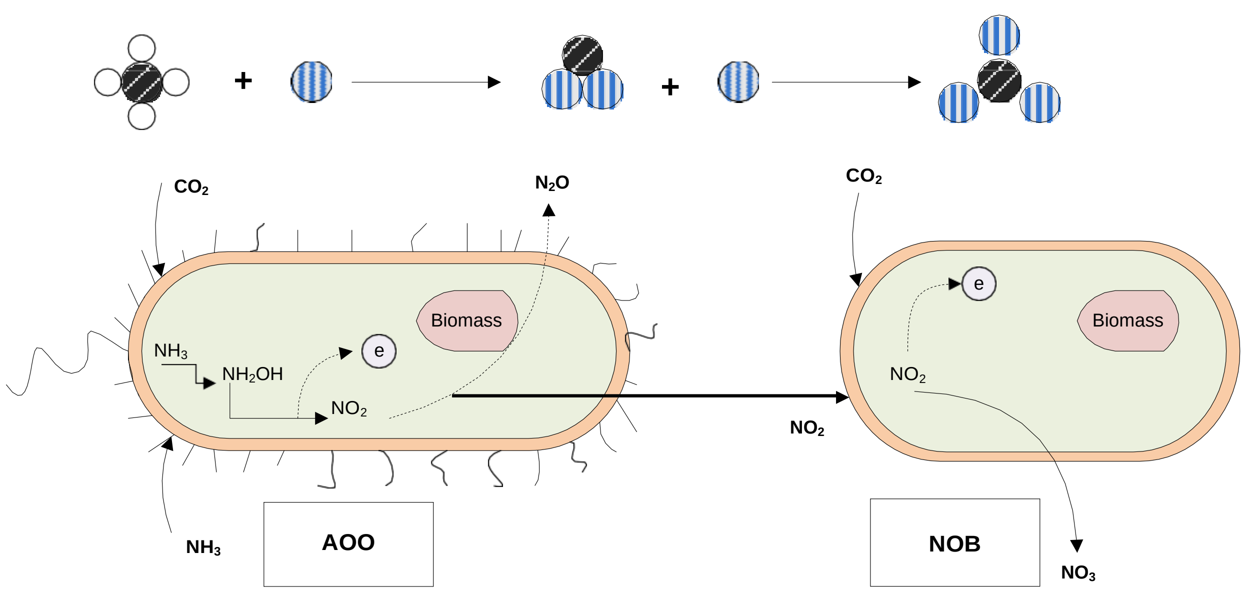


Figure 2- 9: Nitrification process scheme

This process is a biochemical reaction which is reliant on nitrification bacteria, nitrosomonas and nitrobacter. Mentioned microorganisms are autotrophic, which indicates that they consume inorganic carbon (carbonates, bicarbonates and CO_2) as their carbon source. Nitrifiers hold cytomembranes which can be defined as the extensions of cell membrane away from cell wall in the direction of cytoplasm, moreover they are the effective sites for oxidation of ammonium and nitrite ions. Addition of oxygen to ammonium and nitrite ions takes place in presence of enzymes on the cytomembranes of the nitrosomonas and nitrobacter.

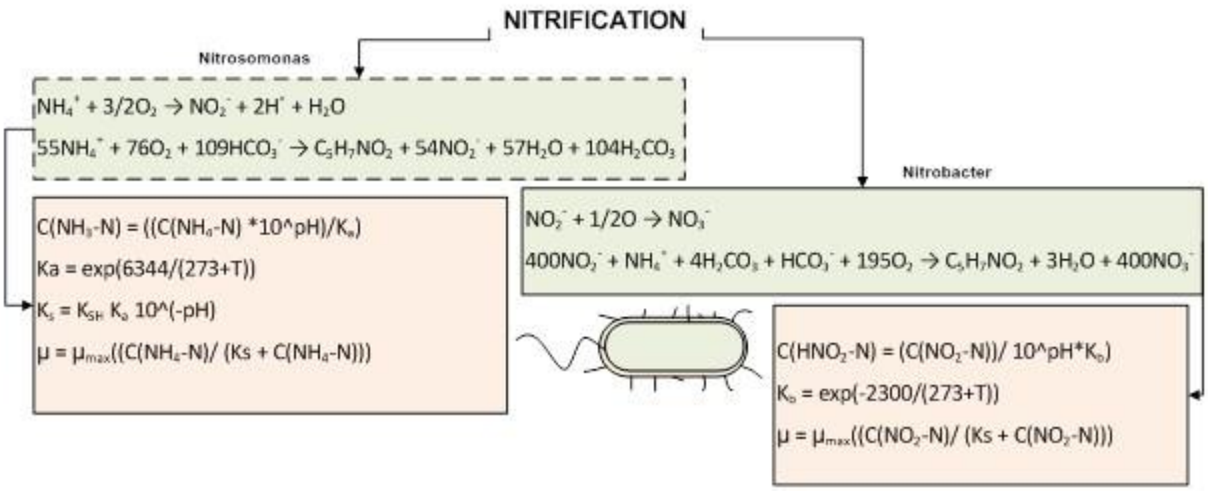


Figure 2- 10: Nitrification process kinetics and stoichiometry

A couple of the effective factors for nitrification are; oxygen concentration, temperature, alkalinity, pH and toxicity. Since nitrifiers are compel aerobes they cannot endure in anaerobic environments. For a tenable and effective nitrification, a DO level of higher than 1.5 mg/l is obligatory. Nitrification microorganisms are sensitive to temperature while the best performance is observed when the process is completed in 30 ˚C (28-32 ˚C). Alkalinity is used as the carbon source for nitrification. Every mg of ammonium ion oxidized, utilizes 7.14 mg of alkalinity as $CaCO_3$. This results in pH drop, which causes a substantial diminution in nitrification. Toxicity is enduring loss of enzymatic activity or else a severe destruction to the cell structure. Nitrifiers are slow growing microorganisms and they are tremendous indicators of toxicity presence (ecos, n.d.).

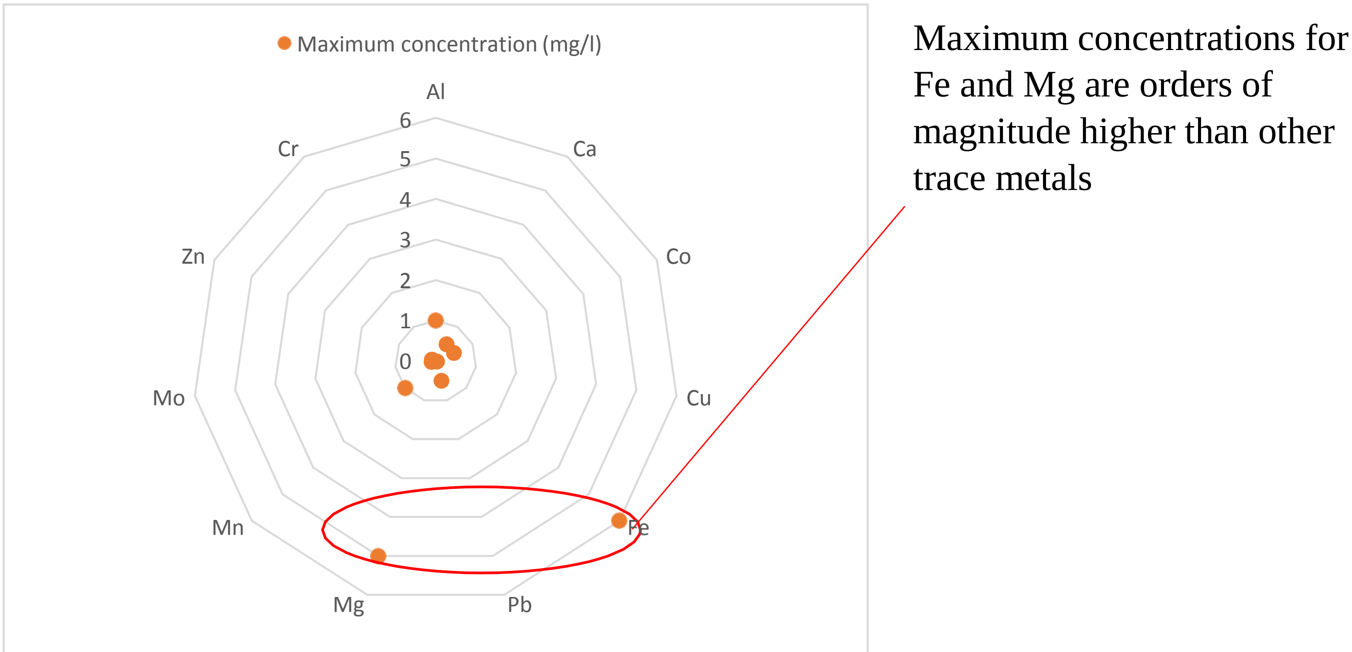
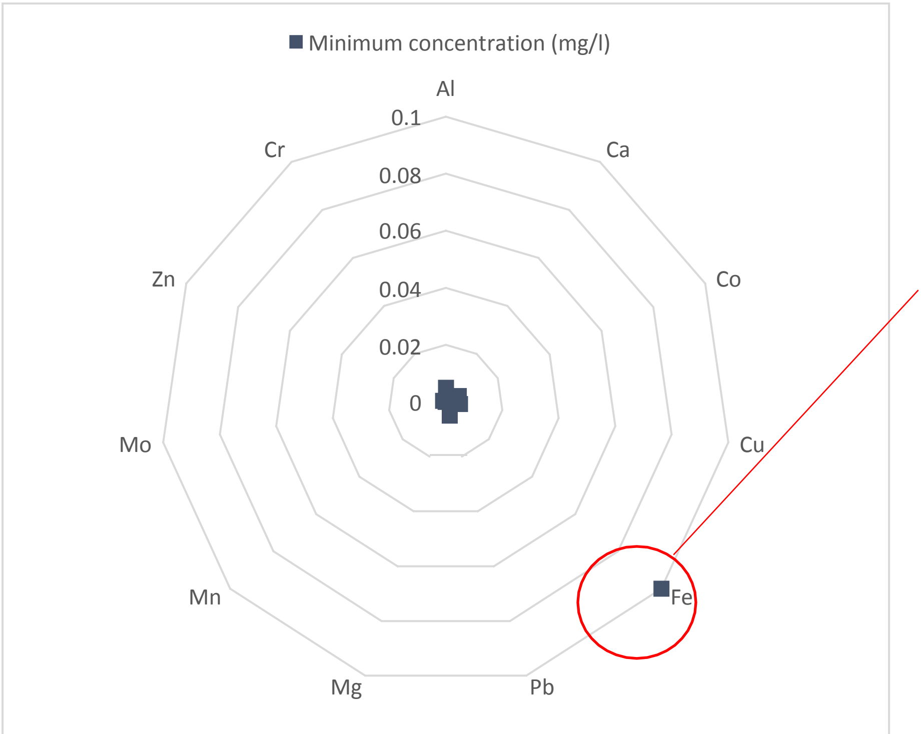


Figure 2- 11: Maximum optimum concentration of metals for nitrification

Different metals have a wide variety of effects on Nitrification process. Metals such as Ca, Cu, Fe, Mg, Mn, Mo and Zn have a considerable positive effect on nitrification if they are used in a limited range. Exceeding the range causes inhibition in the process for Copper, Magnesium, Manganese and Zinc while for Calcium either positive or negative effects become negligible (Cox, 1982) (Wilkie, Goto, Bordeaux, & Smith, 1986).

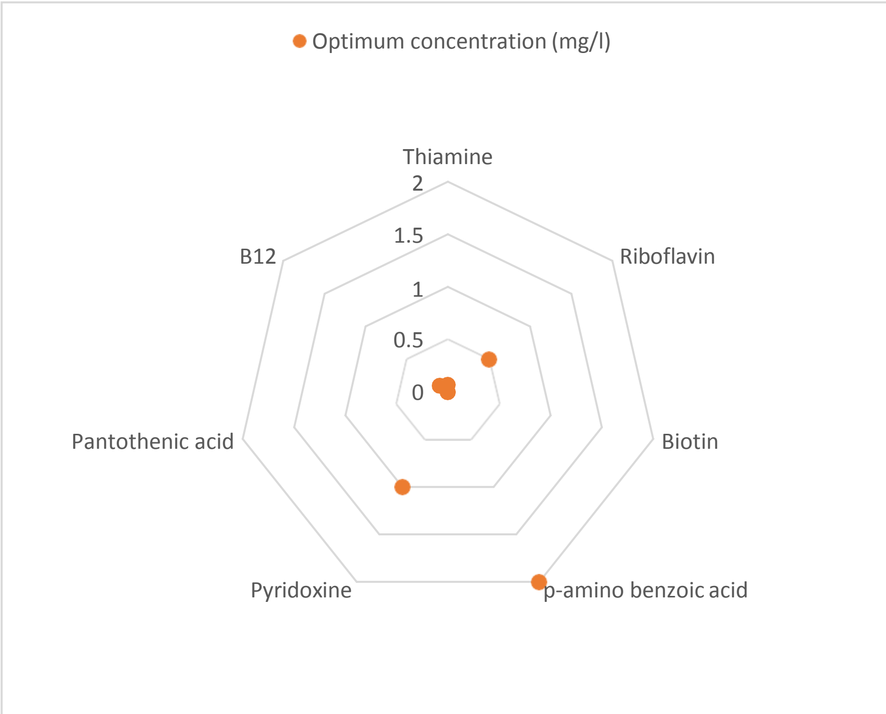
Cobalt seems to be neutral at any concentration either high or low. On the other hand, Chromium has no optimum range of concentration in nitrification and any addition of it obstructs the process. Aluminum and Lead have no specific effect while used in their optimum ranges between 0.005 – 1 mg/l and 0.005 – 0.5 mg/l respectively. Iron acts as a booster when it is added in concentrations from 1.00- 6.00 mg/l (Cox, 1982).



Fe has a minimum concentration of 0.1 mg/l while other trace metals minimum concentrations are approximately 0 mg/l.

Figure 2- 12: Minimum optimum concentration of metals for nitrification

Presence of Cadmium and Nickel ions causes a complete inhibition in nitrification mostly when they are used in high concentrations but this problem can be solved by complexation with aqueous ligands and adsorption of metals directly to the biomass (Hu, Chandran, Grasso, & Smets, 2002).



None of the vitamins have a considerable effect on the Nitrification process.

Figure 2- 13: Effect of optimum concentrations of vitamins on nitrification

Regarding the vitamins, most of the common ones don't seem to have any noticeable effect on the process (figure 2-13). For instance, Thiamine, Riboflavin, Biotin and B_{12} in concentrations around 0.7 mg/l, 0.5 mg/l, 0.0003mg/l and 0.1 mg/l are neutral for nitrification and this is the same for other vitamins such as P-amino benzoic acid, Pyridoxine, Pantothenic acid and Nicotinamide. However, this scheme seems to change for a different group of vitamins. Glutamic acid, Tryptophan, Phenylalanine, Tyrosine and Histidine are considered to be considerably inhibitory for Nitrifiers in a concentration as high as 100 mg/l (Gundersen, 1955).

Besides vitamins and metals, presence of compound such as Thiocyanate, Free cyanide, Ferric cyanide, Phenol, P-cresol causes a negative effect generally in low concentrations. In higher concentrations above 200 mg/l Thiocyanate, Ferric cyanide, p-cresol become neutral to the process (Kim, Park, Lee, & Park, 2008).

The study of nitrification in moving bed biofilm reactor showed that this specific design is suitable for nitrification. To reach the required growth and activity rates of microorganisms in the process, presence of a variety of micronutrients is essential. The necessary trace elements are as following; Iron, Magnesium, Molybdenum, Calcium and Copper (Hem, Rusten, & Ødegaard, 1994).

If low growth rate of nitrifying bacteria is taken into consideration, it is essential to retain the bacteria responsible for nitrification. Immobilizing the bacteria is a considerable method for maintenance of it. The bacteria can be self-immobilized in fluid-bed reactors. The micronutrients used in this reactor design are mainly; Calcium, Magnesium, Iron, Manganese, Zinc, Copper, Nickel, Cobalt and Aluminum (Liu, Wu, Tay, & Wang, 2008).

The suggested additive for the nitrification process is a mixture of metals and vitamins considering both the effects and costs.

Table 2-2: Optimum composition of additives for NIT process

		Chemical properties
	Component	Concentration (%)
Metals	Ca	<0.00005
	Fe	0.00001-0.00006
	Mg	0.005
Vitamins	Pantothenic acid	2.5×10^{-7}
	Biotin + Thiamine	3×10^{-8} + 0.000007

Mg and Fe both have boosting effect on nitrification microorganisms. Both metals have a reasonable range, in which their effect remains positive moreover, Fe has a low price. Ca is also stimulating for the process; however, the range is not as wide as iron and magnesium. Vitamins are not noticeably influential for nitrification process. Pantothenic acid has a slight stimulating effect on nitrifiers this is when, combination of Biotin and Thiamine can similarly improve the process.

Some of the fundamental reasons for the stated effects detected in the nitrification process are listed below:

- **Ni:** Increase in the nitrification inhibition was in a good correlation with increase the Ni^{2+} concentration, Inhibition is strongly correlated with free cation Ni^{2+}. In the procedure of nitrification inhibition, after the uptake of toxic free cations via nonspecific transport systems, the free cations impair the structure and function of cellular proteins. Additionally, they can damage the function of physiologically important ions.

- **Cd:** Increase in the nitrification inhibition was in a good correlation with Cd^{2+} concentration increase.

- **Thiamine:** No influence detected at 0.07 μg/ml in pure culture and mixed culture while, 5 μg/ml caused some inhibition in pure culture and complete inhibition in mixed culture, Pure culture of isolated Nitromonas, Mixed culture of Nitrosomonas and heterotrophic bacteria without addition of glucose. Addition of thiamin with and without glucose towards stimulating the heterotrophic bacteria did not positively affect the nitrification and at 5 μg/L inhibited the nitrification

- **Riboflavin:** No influence was detected at 0.5 µg/ml in pure culture, mixed culture. Addition of the riboflavin did not stimulate the nitrification and at 5 µg/L while it in fact inhibited the nitrification process.
- **Pyridoxine:** considerable effects were not detected at 1 µg/L in pure culture or mixed culture.
- **Biotin:** No influence detected at 0.0003 µg/L in pure culture or mixed culture.
- **Riboflavin:** No influence detected at 0.5 µg/ml in pure culture or mixed culture.
- **B_{12}:** No influence detected at 0.1 µg/L in pure culture or mixed culture.
- **B- vitamin mixture:** No influence on nitrification rates in all cultures (Hu et al., 2002) (Kim et al., 2008).

2.3. Denitrification

Denitrification is a biological process followed up by nitrification, in which nitrate formed in nitrification is converted to nitrogen gas in an anoxic environment. Denitrification is a dependable and steady biological process in wastewater treatment.

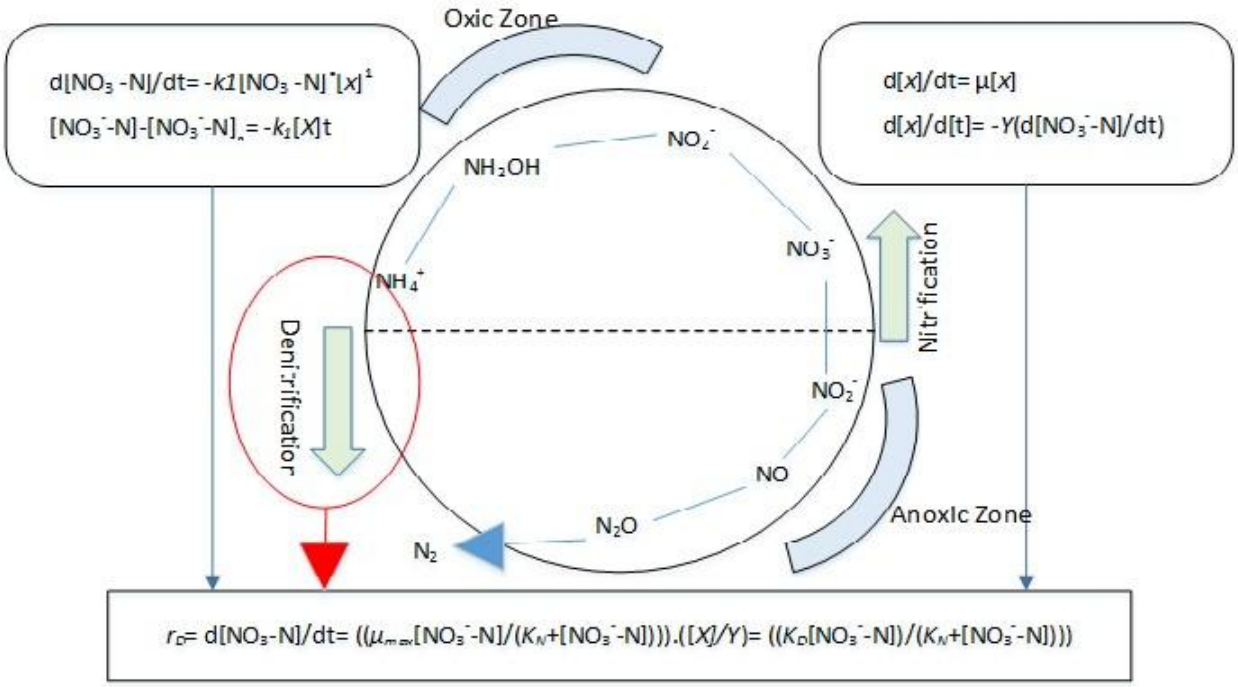


Figure 2- 14: Denitrification kinetics and process scheme

Both anabolic and catabolic pathways can reduce nitrate, however merely the catabolic reaction is referred to as denitrification. Denitrification organism can be either autotrophic bacteria which grow on CO_2 or heterotrophs that can use organics for growth (Foglar, Briski, Sipos, & Vuković, 2005).

There is a wide variety of organism which are capable of consuming oxygen in aerobic metabolism and reduce nitrate in an anoxic metabolism in absence of oxygen. Most of the denitrifiers can use NO_2^- as electron acceptor in place of NO_3^-, while their electron donor differs among different organics (Foglar et al., 2005).

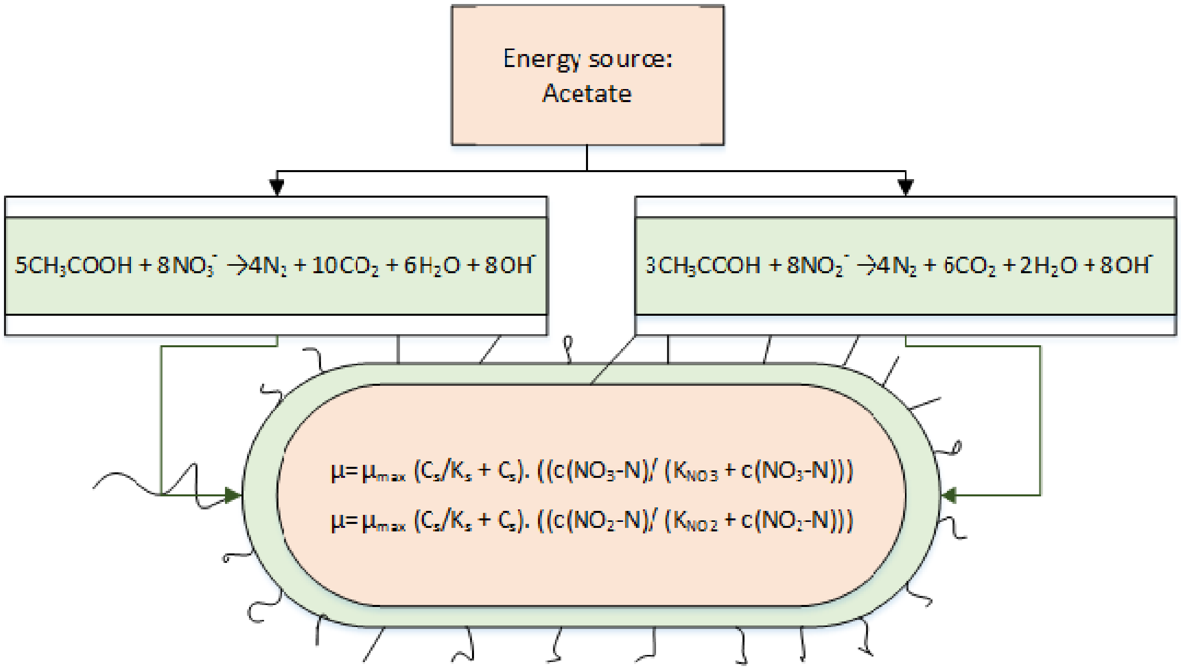
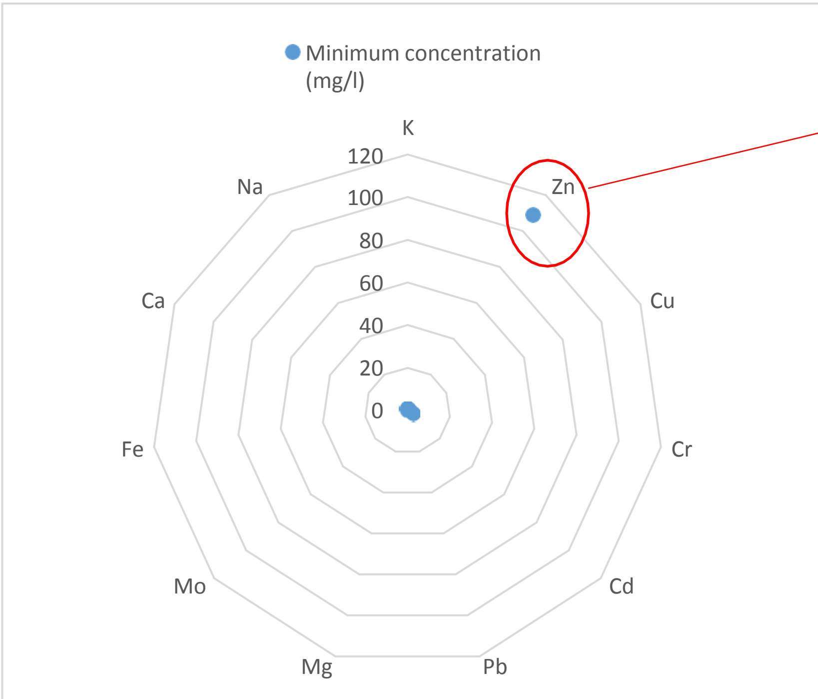


Figure 2- 15: Denitrification stoichiometry and bio-kinetics

Nitrogen compounds are a critical cause of environmental complications, which can result in threats concerning human health and water quality, moreover nitrate is growing in to an enormous apprehension in recent years. With the aim of reducing nitrate in wastewater, biological denitrification is used. The most significant problem is denitrifier's low rates as well as high proportions of time consumption which, requires an efficient denitrification process. Addition of micronutrients is demonstrated to be advantageous for the nitrate removal (Foglar et al., 2005).

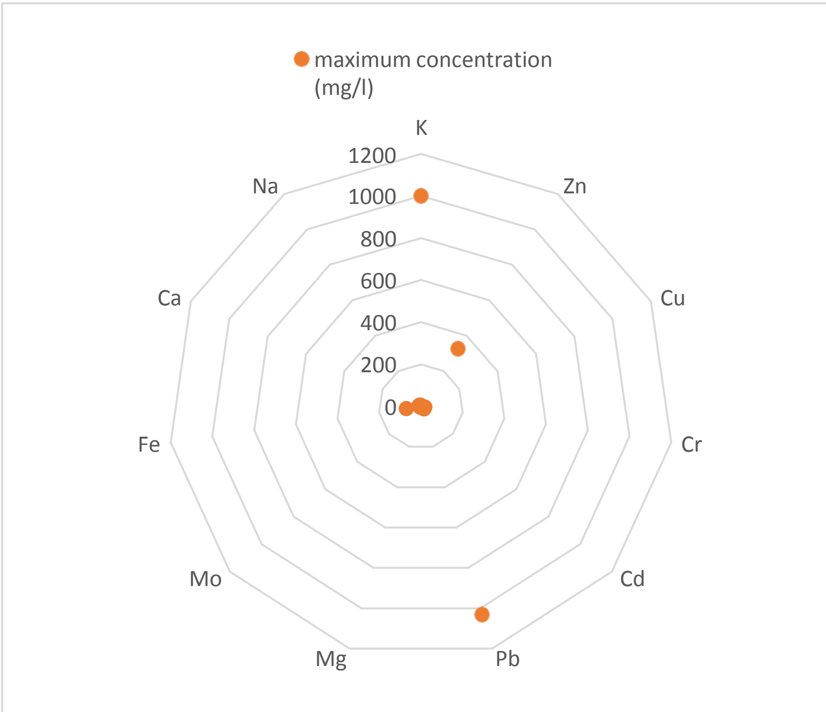
Metals such as Potassium, Zinc, Cadmium, Lead, Magnesium and Iron enhance denitrification significantly if they are used in an optimum range, which differs from a few mg/l to around 1000 mg/l. On the other side Molybdenum has a negative impact either in low concentrations or high concentrations (Magalhães, Costa, Teixeira, & Bordalo, 2007).



Overall trace metal concentrations are higher for denitrification process. Zinc has a high minimum concentration range.

Figure 2- 16: Minimum optimum concentration of metals for denitrification

Exceeding the concentrations above 326 mg/l and 19 mg/l causes inhibition in cases of Zinc, and Cadmium usage respectively (figure 2-16), while it results in Potassium, Lead, Magnesium and Iron to become neutral for the process. Cadmium seems to have no considerable effects independent of its concentration (Cyplik, Grajek, Marecik, & Kroliczak, 2007a) (Magalhães et al., 2007).

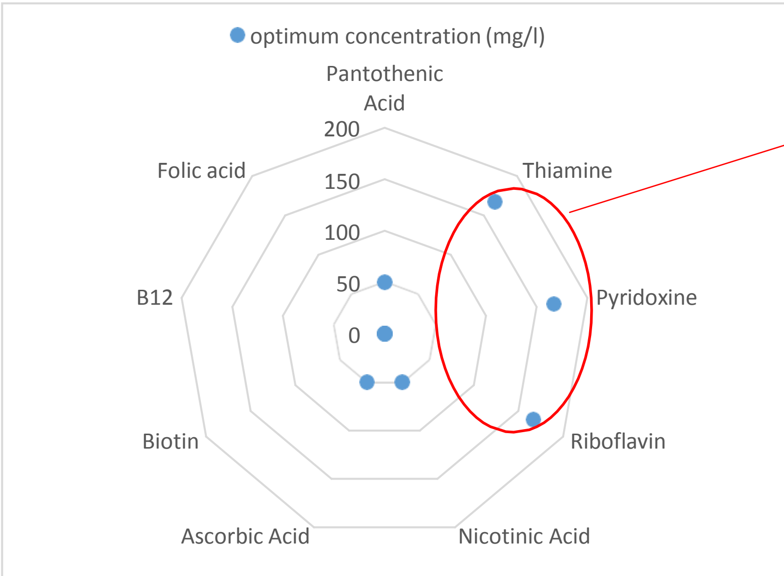


Overall trace metal concentrations are higher for denitrification process.

Figure 2- 17: Maximum optimum concentration of metals for denitrification

Addition of vitamins has a significantly positive effect on denitrification. Based on figure 2-17, 50 mg/l of Pantothenic acid, Nicotinic acid and Ascorbic acid and 167 mg/l of Thiamine, Pyridoxine and Riboflavin results in improvement of the process at a noticeable level. A-palmitate, Biotin, B_{12} and Folic acid enhance the process as well (Sharma & Ahlert, 1977) (Aleem & Alexander, 1960).

Other than metals and vitamins, components such as; Adenine Sulfate, Sodium Glutamate, Yeast Extract, L-Serine, L-Glutamine, L-Glutamic Acid, L-Aspartic Acid and Glucose in a wide range of concentrations can promote the denitrifiers (Sharma & Ahlert, 1977).



Regarding vitamin addition, unlike nitrification process, most vitamins solutions have beneficial effects on denitrification.

Figure 2- 18: Effects of optimum concentrations of vitamins on denitrification

With the aim of studying the effect of trace metals on denitrification the experiments were done in slurries of feed sample and different metal concentrations. The results indicate that excess concentrations of Chromium and Copper can result in inhibition of the process while the utilization of Chromium, Zinc, Copper and Cadmium as trace elements can enhance the denitrification process (Magalhães et al., 2007).

In order to understand the effect of nutrients on denitrification, the bioreactors were first filled with certain amounts of feed and then injected with a suspension of biomass containing microbial culture. Inclusion of Magnesium, Potassium, Iron, Copper and Molybdenum can positively affect the denitrification. Increase in Iron concentration results in more effective denitrification (Cyplik, Grajek, Marecik, & Kroliczak, 2007b).

The suggested additive for the denitrification process is a mixture of metals and vitamins considering both the effects and costs.

Table 2-3: Optimum composition of additives for DEN process

		Chemical properties
	Component	Concentration (%)
Metals	Fe	<0.007
	Mo	<0.0005
	K	< 0.1
	Mg	0.0005
Vitamins	Thiamine	0.0167
	Pyridoxine	0.0167
	Riboflavin	0.0167
	B_{12}	1e-7
	Biotin	0.000002

Fe and Mo are both considerably effective although Mo utilization is high cost. Mg and Ca are also stimulating additives for denitrification moreover, almost all of the vitamins are beneficial for denitrification.

Some of the fundamental reasons for the stated effects detected in the denitrification process are listed below:

- **Fe:** Increase in Fe^{2+} up to 70 mg/dm^{-3} increased the nitrate reduction rate considerably, More increase in Fe^{2+} showed no further increase in nitrate reduction rate, 15% sodium chloride solution, KNO_3 Added to a batch bioreactor under anaerobic conditions, Methanol used as a carbon source in denitrification process, Fe^{2+} is a constituent component of nitrate reductase, Lack of the physiologically active Fe^{2+} results in 60% reduction of the enzyme activity, Fe^{2+} is responsible for conversion of nitrite to nitrogen oxide after combining of nitrites with Fe^{2+}

- **Mo:** Optimal concentration of Mo^{6+} was 5 mg/ dm^{-3}, Higher concentration did not accelerate denitrification, Molybdenum is considered as an electron carrier for nitrate. Molybdenum changes in valence between Mo^{5+} and Mo^{6+}, Activity of molybdenum in denitrification process is stimulated by phosphate through a phosphor-molybdenum complex, in presence of tungsten, molybdenum displaced at the active center of the enzyme leading to deactivation of denitrification process, Molybdenum is important for binding the Flavin with nitrate.

- **Cu:** Optimal concentration of cu^{2+} was 5 mg/dm^{-3}, Increase concentration over 25 mg/dm^{-3} inhibited nitrification strongly.

- **Mg:** Increase in Mg^{+2} from 0.5 to 2.0 g/ dm^{-3} increased the nitrate reduction rate, more increase in concentration up to 5.0 g/dm^{-3} decreased the nitrate reduction rate. Accumulation of Mg^{+2} in Haloferax denitrificans counteracts the high osmotic potential of the environment, Mg^{+2} activates the nitrite and nitrate reductase, a high cation concentration is utilized as a shield against the negative effect on the surface proteins owing to presence of large content of acidic amino acids. A high salt concentrations maintained the weak hydrophobic activity.

- **K:** Increase in K^{+} to 1 g/dm^{-3} showed the maximum nitrate reduction rate. Accumulation of in Haloferax denitrificans counteracts the high osmotic potential of the environment, K^{+} activates the nitrite and nitrate reductase, a high cation concentration is utilized as a shield against the negative effects on the surface proteins owing to presence of large content of acidic amino acids. A high salt concentrations maintained the weak hydrophobic activity.

- **Cr:** Increase in Cr concentration progressively decreased denitrification. Cr inhibited denitrification at primary stage owing to absence of N_2O and NO^{2-}, presenting destructive sensitivity of nitrite reductase towards Cr.
- **Zn:** High concentrations of Zn totally inhibited denitrification. Glucose used as carbon source, KNO_3 as a source of nitrate, Zn affected nitrous reductase that catalyze N_2 production, Nitrite reductase was stimulated by high concentration of Zn led to high N_2O release (Cyplik et al., 2007a) (Magalhães et al., 2007).

2.4. Colloids and particulates

There are three main particle forms in wastewater streams; suspended, colloids and dissolved particles. Suspended particles can differ from large sizes down to as small as 10 µm, while colloidal matters range from 10 nm to 10µm.

Generally, there are simple conventional physical methods for suspended particles removal, such as sedimentation and filtration. Although dissolved molecules require a more complex pathway than physical treatment, colloids removal is the most complex and difficult aspect of conventional wastewater treatment.

Colloidal matters can be divided in to two main types of hydrophilic and hydrophobic colloids. The first group are unstable and include clay particles and non-hydrated metals. Hydrophilic colloids are stable in solution since they carry negative charges on the surface. Similar charges on the particles surfaces result in suspended individual particles in the waste stream (Wakefield, n.d.).

Stability of colloids is usually defined through zeta potential (z_p). Zeta potential contributes to the repulsion forces between colloidal particles, therefore higher magnitudes of z_p results in more stabled solutions. In low z_p conditions the particles are stabilized and tend to aggregate (Wakefield, n.d.).

Colloids removal requires destabilization of the small particles with the intention to create larger and heavier flocs. Subsequently the larger sized particles can be removed through conventional physical treatments.

An advantageous alternative for activated sludge process is membrane bioreactor. This process has less footprint and sludge production rate due to high rate of biomass maintenance in the bioreactor. MBR's are capable of handling influent fluctuations while the effluent of the process has the potential of being reused for non-potable purposes. The rate of nitrification is also high in the reactors as a result of high retention times (Barjoveanu & Teodosiu, 2010).

Regardless of all stated advantages, a very critical barrier towards membrane bioreactor implementation is membrane fouling. This negative factor leads to flux decline, frequent membrane cleaning, replacements and higher cost rates. One of the main causes of fouling in MBR's is presence of colloids and supra-colloids in the waste solution. In specific studies approximately 50% of the membrane fouling was contributed to colloidal matters in the wastewater stream (Wakefield, n.d.).

2.5. Conclusion

Biological treatment processes are the core unit operation in the entire wastewater treatment plant. The microorganisms involved in the process, breakdown the organic matters available in the wastewater stream and result in mineralized final products in the effluent. The colloids and soluble organics are aimed to be converted to particulate biomass and subsequently separated through sedimentation.

There are several different biological systems employed for wastewater treatment, while all processes are based on a similar fundamental mechanism; microorganism's growth and maintenance by organic and inorganic material utilization.

Support of microbial growth in biological systems require a diversity of factors such as appropriate nutritional balance. Nutrition supplementation depends mainly on the microorganism's type and nature but the fundamentals are the same amongst all.

Chapter 3: Effect of trace metals and vitamins addition on biodegradability in BOR process

3.1. Introduction

The key subdivision for organic matters in wastewater is established based on biodegradability. Biologically inert materials pass through the activated sludge system deprived of the minimum variations in form. Two acknowledged fractions are soluble and particle inert, in which soluble matters leave the process at the identical concentration in the influent, while the suspended portion removal is completed through sludge wastage (Henze, Grady, Gujer, Marais, & Matsuo, 1987).

Biodegradables can be distributed in to two core sets; readily and slowly biodegradable matters. The initial group, which are predominantly simple molecules, are treated as solubles while the slowly biodegradables comprised of complex molecules, are treated as particulates. While the slowly biodegradables are removed by flocs in suspension, readily biodegradable portion of the substrate is the main source for heterotrophic bacteria growth (Clark & Stephenson, 1998a).

Heterotrophic bacteria as a subdivision of microorganisms involved in wastewater treatment processes, necessitate a range of assorted macro and micronutrients. There are ten indispensable macronutrients for cell synthesis and comprise at least 95% of the cell; carbon, oxygen, hydrogen, nitrogen, sulphur, magnesium, potassium, phosphorus, calcium and iron. The stated metals are required for enzymatic activities and protein production, membrane transport, enzyme cofactors, ribosome's and cell membrane's stabilization and cytochrome's synthesis (Clark & Stephenson, 1998a).

A variation of trace metals is compulsory for microbial life; copper, nickel, cobalt, zinc, manganese, boron, vanadium and molybdenum. There might be insufficient nutrient source owed to missing nutritional components in wastewater or unavailability of the existing nutrition to the biological cells because of chemical reactions or precipitation. The nutritional requirement is measured through interactions between the bacterial growth rate, maximum yield and the substrate utilization rate, moreover the maximum yield is a ratio of formed cell mass to consumed substrate mass (Burgess, Harkness, Longhurst, & Stephenson, 2000).

In an ideal environmental conditions and balanced vital elements concentrations, there is an increase in yield coefficient, additionally known yield coefficient leads to measurable nutrient trace elements requirements in activated sludge process;

Y= (mass of bacteria produced)/ (mass of BOD used)

Equation 1: Yield coefficient definition

(Clark & Stephenson, 1998a)

Based on the subsequent table (table 3-1) the required concentrations for trace elements are shown per a liter of activated sludge. One of the most recognized and extensive biological wastewater treatments both for municipal and industrial wastes is activated sludge treatment process. This conventional process relies on mixed microbial suspension in aerobic conditions (aeration boosts the bacterial growth) while present microorganisms oxidize the organic matters in the influent, furthermore the products of the process are biomass and oxidation products. It is important to notice the significance of organic loading, hydraulic loading and biomass growth in nutritional requirements (Burgess, Harkness, et al., 2000).

Table 3-1: Effect of specific trace metals on activated sludge process

Trace metal	Optimum concentration range (mg/l)
K	0.8-1.5
Na	0.5-2
Ca	0.4-0.7
Mg	0.4-0.7
Cl	0.4-0.7
Fe	0.1-0.4

The ultimate objective of biological wastewater treatment processes is placement of carbon as a limiting factor and hence balancing the nutrition to achieve low carbon concentrations in effluent (Burgess, Harkness, et al., 2000).

The primary performance parameters measured in activated sludge are the effluents BOD and suspended solids concentrations, this is while recent legislations resulted in specific limitations for COD concentrations in the effluent. Biodegradation is mainly affected by environmental factors such as nutrient availability; additionally, nutrient augmentation refers to addition of micronutrients which increase the co-metabolism and induce the degradive genes (Burgess, Harkness, et al., 2000)(Jefferson et al., 2001a).

As stated previously the general aim of the biological processes is achievement of low rates of BOD by placing carbon as the limiting factor. Support of all bacterial growth and maintenance requires a variety of both macro and micronutrients. Addition of trace metals to the biomass is an extremely critical task. Deficiencies can be corrected by mixture solution addition; however, salts and complexes may be an unsuitable source of nutrients for bacteria. The adding process is very sensitive due to process inhibition caused by high and excessive concentrations of trace metals (Burgess, Harkness, et al., 2000).

Co addition to activated sludge process resulted in higher rates of B_{12} synthesis while excessive concentrations of the trace metal caused less purification, nitrification inhibition, flocculation and lower effluent qualities (Clark & Stephenson, 1998b)(Jefferson, Burgess, Pichon, Harkness, & Judd, 2001b).

Since there is a variety of diverse species in activated sludge, addition of trace metals can suppress a specific category while benefiting other species. Toxicity of heavy metals in activated sludge process is mainly based on the metal's specie and concentration while other environmental factors; pH, sludge concentrations, influent strength, etc. are less critical. Excessive concentrations of heavy metals block the enzymatic systems and interfere with vital cellular metabolisms.

As activated sludge process is very sensitive to trace metals concentration and type, the determination of the exact amount of each specie is very difficult, this leads to consideration of other additives such as vitamins as biological supplements for wastewater treatment (Clark & Stephenson, 1998b).

Table 3-2: Effect of trace metals and vitamins optimum concentrations on BOR process

	Supplements	Optimum **concentration range** mg/l	**Supplement effect**	**Exceed the concentration range**
Metals	Ca	0.4-1.4	+++	NA
	K	0.8-3.0	+++	NA
	Fe	0.1-0.4	+++	NA
	Mg	0.5-2.0	+++	---
	Mn	0.01-0.05	+++	NA
	Cu	0.01-0.05	+++	---
	Al	0.01-0.05	+++	---
	Zn	0.1 -1.00	+++	NA
	Mo	0.1-0.7	+++	NA
	Co	0.1-5.0	+++	---
	Na	0.5-2.0	+++	NA
	Cr	-	*	---
Vitamins	Biotin	0-1.0 µg/l	+++	
	Niacin	0-1.0	+++	
	Thiamine	0-1.0	+++	
	Lactoflavin	0-1.0	+++	
	Pyridoxine	0-0.01 µg/l	+++	
	Pantothenic acid	0-1.0	+++	

	B_1	0.3-1.2	+++	
	B_2	0.5-2.0	+++	
	B_6	0.3-10	+++	
	B_{12}	0.5 µg/l	+++	

GUIDLINES

+++: Significant effect
++: Beneficial
+: Weakly stimulating
*: No effect
-: Weakly inhibitory
--: Inhibitory
---: Very inhibitory
----: Complete inhibition

Vitamins as active elements can aid the purification, sludge accumulation, process disruption and energy usage. Based on former studies, although vitamins can benefit the cell growth rate in industrial wastewaters, there was no major escalation observed in municipal wastewaters. Single vitamin supplementation did not affect the enzyme activity, nonetheless a full vitamin complex led to significant enzymatic activities (Clark & Stephenson, 1998a)(Selimoğlu, Öbek, Karataş, Arslan, & Tatar, 2015).

Furthermore, addition of vitamins did not result in any noticeable increase in degradation rates, however since different vitamins result in dissimilar effects, it is essential to study them independently.

Lactoflavin (B_2) is an essential growth factor for certain bacteria, while riboflavin has a significant role in cell metabolism. The former studies indicate that vitamin B_6 is a fundamental growth factor for lactic acid bacteria, on the other hand there are no specific requirements for folic acid and adermine in activated sludge. Pantothenic acid as a member of group B vitamins, is a growth matter and respiration stimulant, it is important to recognize the effectiveness of pantothenic acid in presence of other group B vitamins (Clark & Stephenson, 1998a)(Selimoğlu et al., 2015).

Table 3-3: Advantages and disadvantages of additives for activated sludge process

Additive	Advantages	Disadvantages
Trace metals	Boost in vitamin B_{12} synthesis	Toxic effects in excessive concentrations, reduced effluent qualities and inhibitions in flocculation
Vitamins	Enhancement in enzyme activity, reduced sludge bulking	Sensitive concentration determination
Vitamin group B	Vital for cell growth and metabolism	

Biodegradability tests can be performed to monitor the COD removal efficiency, oxygen uptake, BOD removal rates as well as solids removal, in addition, these tests can indicate the potential of micronutrients for maximized removal rates.

Microbial respiration is a biochemical term in which organic or inorganic compounds are electron donors whereas inorganics have the role of electron acceptors. This process is a ATP (adenosine triphosphate) generating metabolism, in addition ATP is produced while electrons are transferred from the electron donor to acceptor. Present microorganisms transform the generated energy of intramolecular bond of electron donor to phosphate bonds in ATP. This energy is further used for cell growth (anabolism), maintenance and reproduction (Clark & Stephenson, 1998a).

In the oxidation process referred to as respiration, electron donor and acceptor are converted to oxidized and reduced forms respectively. Heterotrophs convert carbonaceous donors with presence of oxygen as electron acceptor, in addition the products of the oxidation-reduction are carbon dioxide and water.

Ammonium, nitrite, ferrous and sulfide serve as electron donors with presence of oxygen as electron acceptor by means of autotrophs such as nitrifiers (AOB and NOB), iron oxidizing bacteria and sulfide oxidizing bacteria.

Non-aerobes can also use inorganics such as NO_3^-, NO_2^-, SO_4^{2-} and CO_2 as electron acceptors. These processes are either anoxic or anaerobic processes. In general, the metabolisms involved in respiration are catabolic reactions (Clark & Stephenson, 1998a).

The energy essential for cell growth and maintenance such as reproduction, cell mobility and osmotic activity is made by microbial respiration. Overall, on a weight to weight base merely half of the oxygen respiration is connected to the biomass growth through yield. As stated previously in activated sludge process oxygen consumption is linked to nitrification and microbial oxidations additional to the carbonaceous substrate removal.

Respirometery is defined as clarification of inorganic electron acceptor biological consumption rate under explicit experimental circumstances. The device used for respiration rate is referred to as respirometer. These devices range from simple manually operated devices with a certain sensor, to complex automatic devices (Vanrolleghem, Olsson, & Spanjers, n.d.).

The main working basis of respirometers is dissolved oxygen concentration (DO) measurement by means of a DO mass balance. The DO mass balance over the liquid phase is:

$$d(V_L.SO_2)/dt = Q_{in}.SO_{2in} – Q_{out}.SO_{2out} + V_L KL_a. (SO_2*-SO_2) – V_L.r_{O2}$$

Equation 2: Oxygen mass balance equation

in which SO_2 is the DO concentration in liquid phase while the starred parameter is the saturated DO concentration. KL_a can be defined as the oxygen mass transfer coefficient while Q demonstrates the flows in and out of the system. r_{O2} is the respiration rate of the biomass portion of the liquid phase (Vanrolleghem et al., n.d.).

A simple approach is the static gas and liquid mass balance in liquid phase. The two initial parameters cancel out due to no flows in and out of the system and the third component represents the mass transfer between the liquid and gas phase which is eliminated in static liquid and gas condition:

$$dSO_2/dt = -r_{O2}$$

Equation 3: Respiration equation for static gas and liquid phases

The mass balance shows that respiration rate can be obtained by DO concentration decline measurement. Most respirometery tests include different measuring arrangements such as sensors; to measure the oxygen concentration. In simple laboratory instruments (BOD tests) DO is calculated by a titrimetric or photometric technique (Vanrolleghem et al., n.d.).

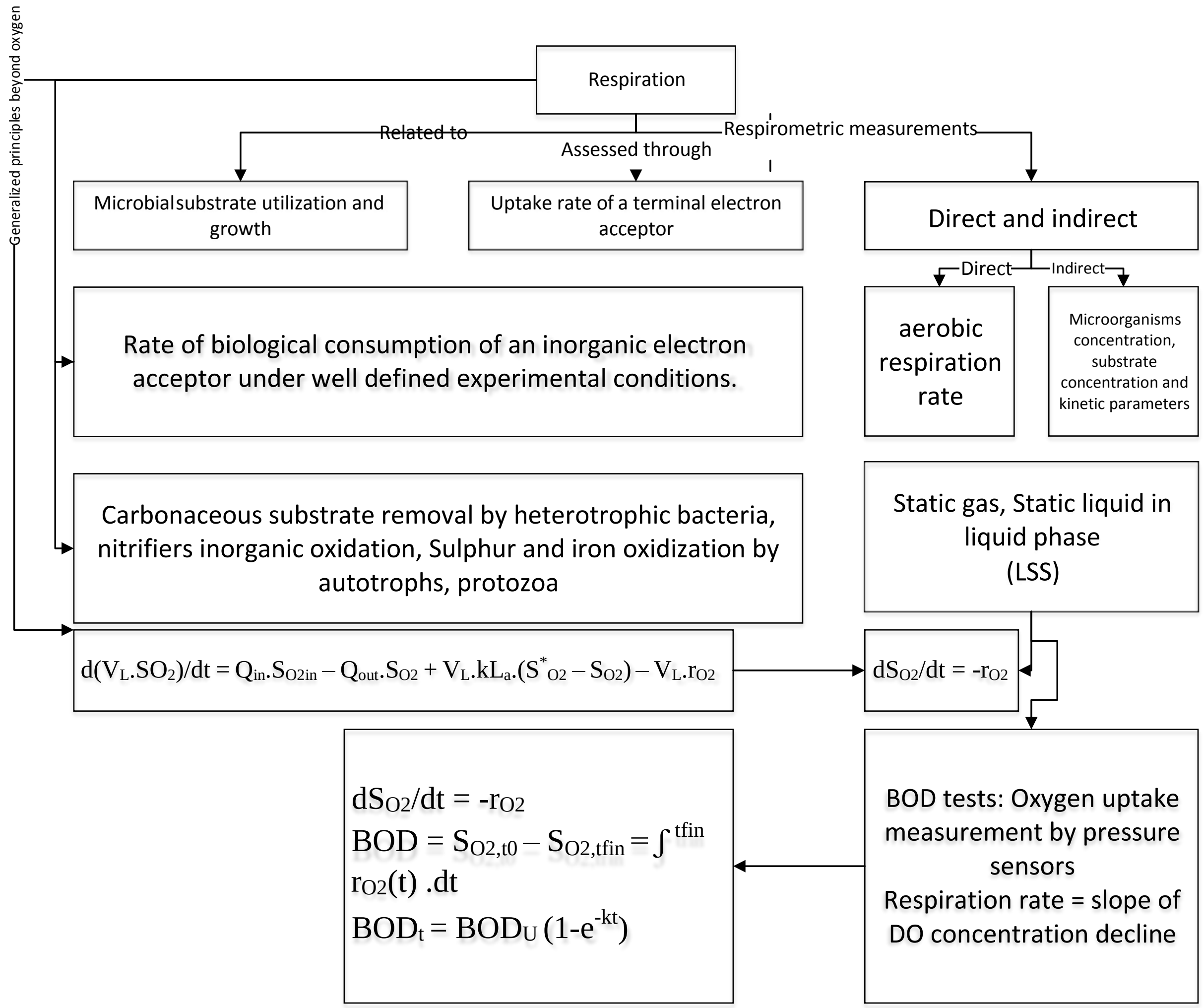


Figure 3- 1: Respiration principles and categorization

In the LSS principal (figure 3-1), since there are no gas or liquid flows, there aren't any requirements for additional devices such as pumps, aerators, etc. the BOD test evaluates the biodegradable organic matter concentration in wastewater to assess the design based on organic removal matter. Bacterial consumption of oxygen is specified through this test which shows the bio-degradation of organics in the influent. Total BOD consists of both carbonaceous and nitrogenous biochemical oxygen demands (Vanrolleghem et al., n.d.).

3.2. Materials and methods

With the aim of concluding the effect of the biostreme and vitamins solution on the activated sludge process, and biological organic removal (BOR) process, a series of diversified biodegradability batch tests were conducted.

The system employed for the experiments was BOD Trak II, Hach which is one of the most common and straight forward methods of BOD measurement. In this system, the BOD is measured over a physical pathway with the elimination of any requirements for chemical measurements. All the environmental variables, such as; experimental temperature, wastewater composition, aerated water, dilution factor and nutrient buffers were stable all through the tests, besides supplementary trace metals and vitamins concentrations.

The data of the organic removals and BOD fluctuations versus time, from the study, were automatically saved and put to graphs for the period of the tests. The data were available at all times during the test and could be simply reviewed thru the BOD evaluation. After five days of experiment, the samples were taken from each test bottle and the COD fractions and solids were analyzed. To present a comprehensive and in depth justification of the materials and methods the system is entirely described in nest subsections; laboratory apparatus, test startup, data collection and analytical measures, approaches of analysis.

3.3. Laboratory apparatus

The setup consisted of number of separate bottles. A specific measure of sample, seed and water were placed in the amber bottles which were connected to the instrument. Each sample bottle was connected to a sensor which monitored the pressure in the closed space of the bottle all through the test. (figure 3-2)

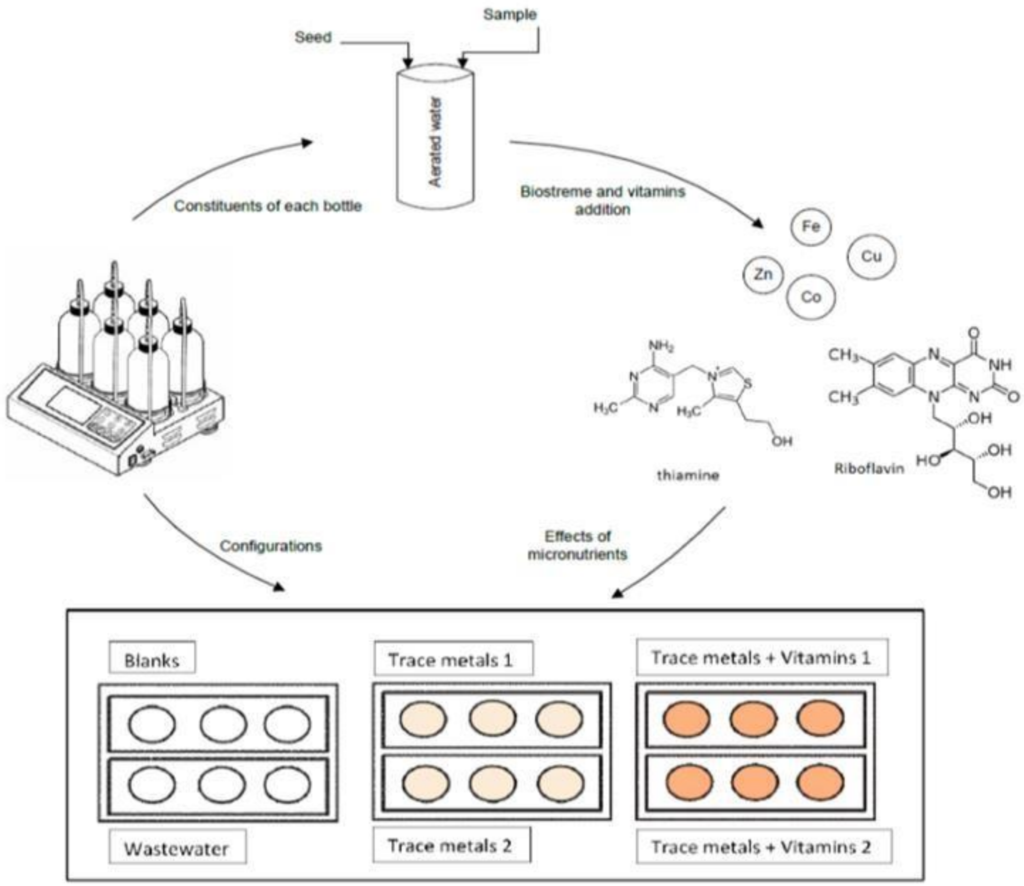


Figure 3- 2: Experimental setup using additives in BOD batch test and configuration

There was a quantity of air overhead of the liquid phase in the test bottles which contained 21% oxygen. Bacteria present in the sludge sample consumes this dissolved oxygen during the experimentation to oxidize the organic matters which, results in a descent of the air pressure. The pressure drop in the bottle is directly associated with the BOD, since it determines the amount of oxygen consumed by the bacteria remaining in the sample. All the bottles were completely mixed perpetually with a magnetic stirrer, the continuous stirring resulted in further oxygen supplies for the sample and more exposure to food for the bacteria. The adequate agitation maintains rapid oxygen transfer from liquid phase to the gas phase. Each device consists of six, 492 ml separate bottles. The depth and height of each bottle is 10.3 and 3.9 inches. Method used for the tests was standard in which, sample, seed and dilution water added to the bottles were 110 ml, 10-35 ml and 350 ml, respectively. The intention behind indicating volumes for each parameter is the BOD range specified in the device. The anticipated BOD range for this precise experiment was in between 0 to 350 mg/l which lead to the stated volumes.

All the tests were automatically ended after 5 days of operation. The data were gathered as a graph which presented the BOD fluctuations versus time. (see section 3.6)

3.4. Test startup

The tests were prepared in three different divisions. The primary sector was done with the utilization of municipal wastewater as the sample, and RAS, in place of the seed in the experiment. The sample was municipal wastewater in both remaining experiments, while the seed altered to recycled AS collected from a laboratory activated sludge system and yeast.

The AS was collected from a laboratory activated sludge system, which consisted of six aeration bioreactors, six clarifiers and twelve effluent and influent tanks. The system was fed daily and continuously with real wastewater collected from Humber treatment plant and mimicked the treatment plant regarding the design factors. The AS was collected from the bottom portion of the clarifier in which the sludge has been settled.

Each bottle was suffused with certain volumes of sample, seed and aerated distilled water.

Table 3-4: Volumes of BOD bottle components

BOD range (mg/l)	Sample volume (ml)	Seed volume (ml)	Final volume (ml)	Dilution factor
0 to 350	110	20	130	1.45

The activated sludge used in the test, either directly as RAS or indirectly from the laboratory sized activated sludge system, as well as the real wastewater were all collected from Humber municipal wastewater treatment plant (second largest facility of the city), Toronto, Ontario.

The aerated distilled water for the batch BOD test was prepared two days before the test startup. A mixture of distilled water and certain amount of nutrient were aerated with air tubes for a 48-hour period. The samples and seeds were brought to ambient temperature before each test, 19 to 21 ˚C., furthermore, the wastewater used in the experiment as the sample was completely homogenized with no large settled or buoyant solids inside of it. Certain amounts of sample, seed and aerated water were added to the bottle, and eventually two potassium hydroxide pellets were added to the seal cup on top of the bottle.

Assorted concentrations of vitamins and trace metals were supplemented to the batch system tests. Each configuration was done in triplets, with the purpose of ignoring errors or else any out of range data in the test.

In each test cycle, blank tests were implemented, to exclude the dilution water's effect from the results. By this specific set up, effect of trace metals only and in a mixture with vitamins was studied. Nine different setups were studied in this research.

Table 3-5: Different configurations of BOD tests

Study / seed	Trace metal 1 ppm/L wastewater	Trace metal 2 ppm/l wastewater	Trace metals + Vitamins 1 ppm/l wastewater	Trace metals + Vitamins 2 ppm/l wastewater
1 / RAS	100	200	200 + 100	200 +200
2 / AS	100	200	200 + 100	200 + 200
3 / yeast	100	200	200 + 100	200 + 200
4 / RAS	100	500	500 + 100	500 + 200
5 / AS	100	500	500 + 100	500 + 200
6 / yeast	100	500	500 + 100	500 + 200
7/ RAS	500	-	500 + 100	-
8/ RAS	-	-	0 + 100	0 + 500

As specified in table 3-5 the first six tests were done with utilization of biostreme individually and in a combination with the vitamin's solution in various concentrations. Test 7/RAS was an assessment of the effect of both the food to microorganism's ratio and the additional supplementation of nutrients on the organic removal, while the final test was operated solitary with singular vitamins as well as vitamin's mixture.

3.5. Data collection and analytical measures

Biochemical oxygen demand can be described as a parameter expressed in mg/l or ppm which determines the amount of oxygen that the bacteria uptake from the water during organic matter oxidization. The BOD measurement is one of the basic determinations of water pollution degree.

The municipal wastewater's (sample), total and soluble COD and VSS were measured before the test's initial startup. Both COD and VSS determinations were prepared on unfiltered samples with standard methods for wastewater analysis. All the data were automatically collected and imported in to spread-sheets. The dilution factor in these specific experiments was 1.45.

For the seeded samples, BOD of the seed must be known. The BOD calculations in such tests is as following:

$$BOD = \frac{(BOD\ of\ diluted\ sample) - (f\ of\ seed \times BOD\ of\ seed)}{f\ of\ sample}$$

Equation 4: BOD correction equation

After data correction, graphs indicating the alterations of BOD versus time were conducted and compared.

3.6. Results and discussion

3.7. Recycled activated sludge (RAS)

There are various responses to altered concentrations of supplements, with use of the recycled activated sludge, attributable to the mixed culture characterization. Concentrations of trace metals and vitamins, as well as, stimulation in the biomass is detected through increasing the yield of the process.

In this study, the effects of trace metals and vitamins in numerous concentrations were evaluated by assessment of BOD alterations set against time.

The study of both singular and mutual effects of metals and vitamins on recycled activated sludge was accomplished underneath mentioned environmental circumstances. All the tests were prepared in trios. Included below is a demonstration of the noteworthy outcomes of the test.

Trace metals. The effect of specific metals- Co, Cu, K, Mn and Zn- were studied through biodegradability batch tests. In each test cycle, three test bottles were filled with precise volumes of RAS as the sample and aerated distilled water. The predetermined assessments were known as blank tests used in further calculations. Each set of three batch bottles were loaded with exact identical components, excluding any additional supplements such as metals and vitamins. Control tests served as standard data for additional comparisons between advantageous and detrimental effects of metals and vitamins on microbial functions. The biochemical oxygen demand curve against time was characterized in a five-day period (Figure 3-3). The effect of various concentrations of trace metals on RAS is shown in the following chart.

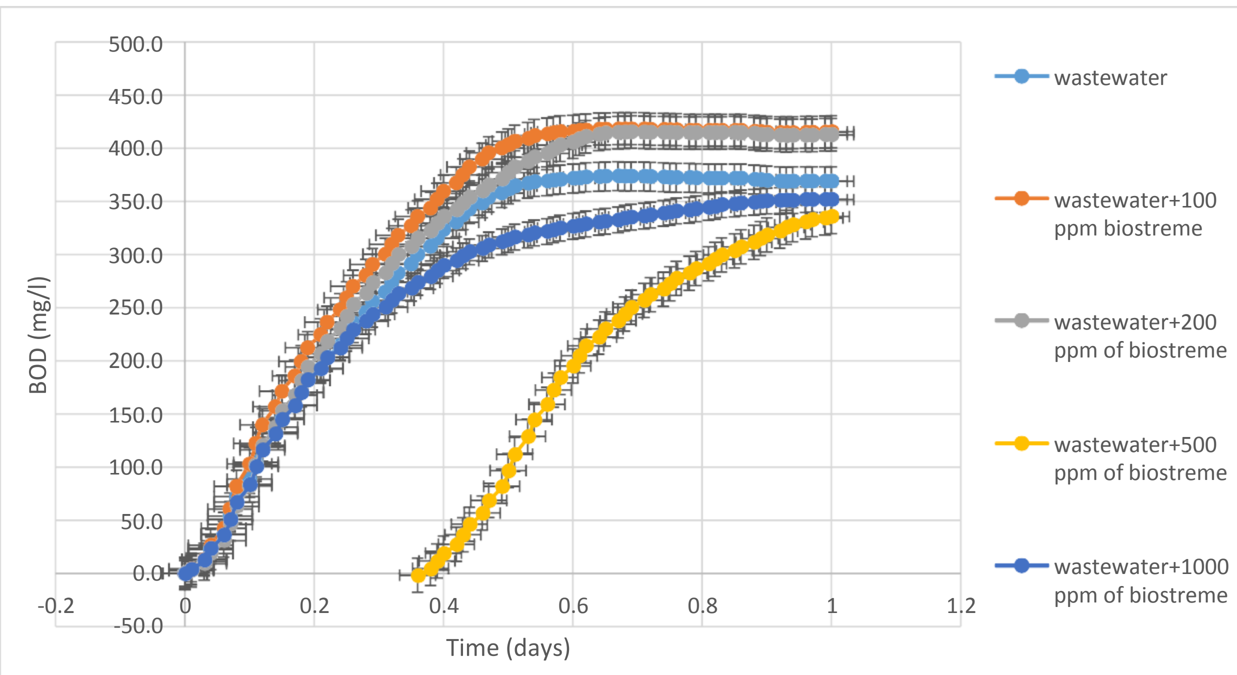


Figure 3- 3: Effect of biostreme addition on BOD tests done with RAS as the seed

It should be taken to consideration that in figure 3-3 the data are not normalized based on the specific characteristics of real wastewater and recycled activated sludge utilized in different test cycles. The graph indicates the fluctuations of the BOD in mg/l against time, considering addition of several different concentrations of trace metals. Figure 3-4 emphasizes the specific period of the test duration before the stationary phase.

The highest yield before data correction is for the test with 100 ppm of biostreme while the lowest slope is for the bottle containing 500 ppm of the trace metal's mixture.

After further data normalization with the recycled activated sludge chemical oxygen demand and volatile suspended solid, the data was compared in mgCOD/gVSS.

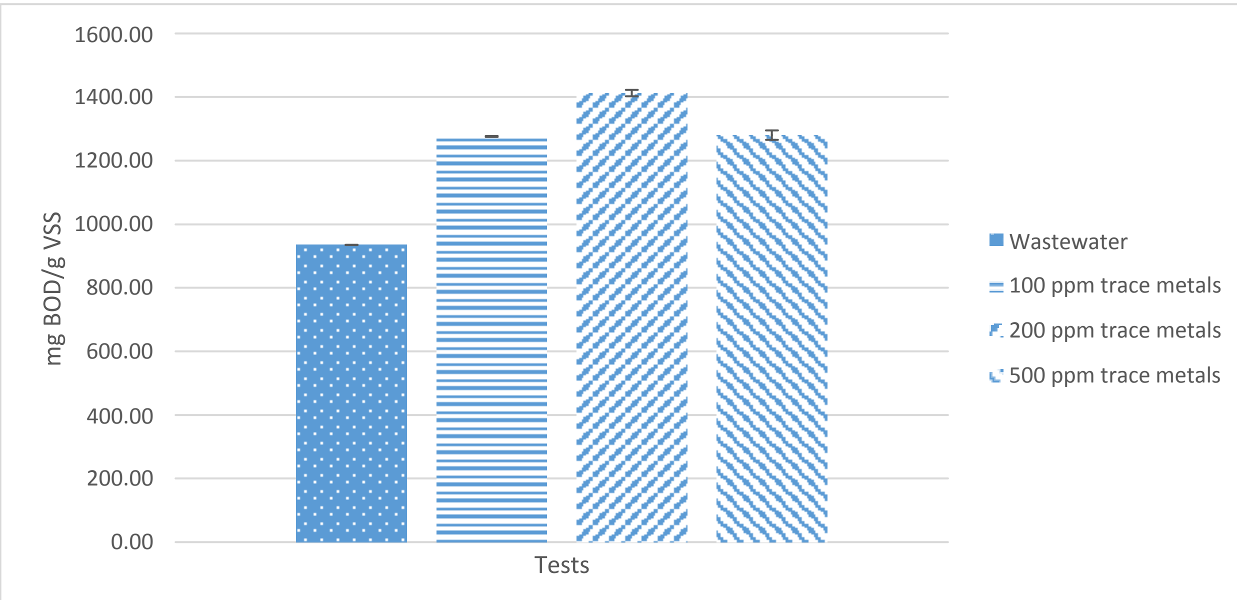


Figure 3- 4: Effect of biostreme addition on BOD tests done with RAS as the seed

The uppermost amount of mgCOD/gVSS observed in all tests is when the wastewater was supplemented with 200 ppm of trace metal mixture, while addition of greater quantities of trace metals lowered the yield of the organic removal.

Trace metals and Vitamins. The effect of vitamins on biological organic removal from recycled activated sludge was studied in combinations with trace metals. The time course data for the effect of different vitamin concentrations ranging from 100 to 200 ppm are shown in the figure 3-5.

Figure 3-5 is based on the data collected from the BOD trak II. The blank is excluded from the data, in addition, each BOD concentration is corrected based on the different sample and seed volumes in individual tests. Figure 3-5 shows that the BOD range is higher than the control (WW) test, when the samples are supplemented with 200 ppm of trace metals and both 100 and 200 ppm of vitamins.

The initial BOD results in some of the tests including 500 ppm of trace metals and both 100 and 200 ppm of vitamins are negative. The reason may be air suction at the beginning of the test due to negative air pressure in the sealed lid.

For better comparisons between different configurations, the results were normalized by COD and VSS of the wastewater. The normalized data are compared in figure 3-5.

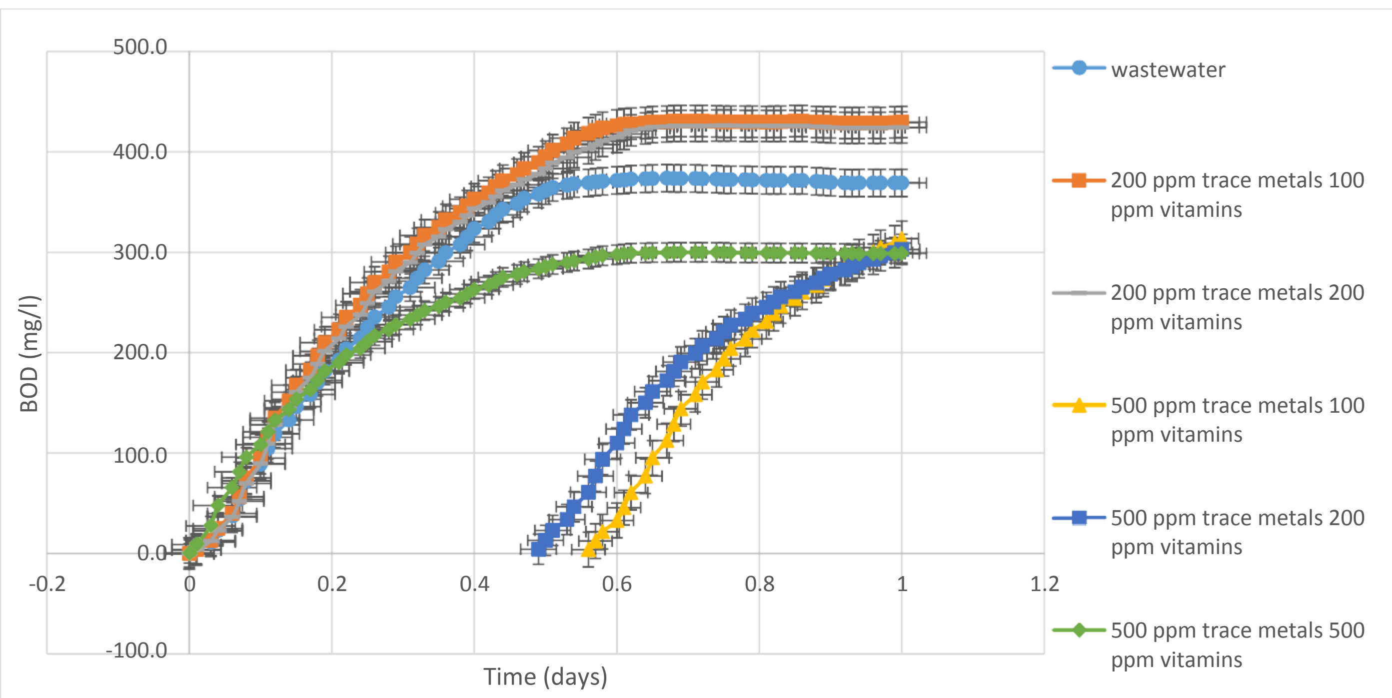


Figure 3- 5: Effect of biostreme in mixture with vitamins solution addition on BOD tests done with RAS as the seed

Addition of higher concentrations of trace metals resulted in lower yields (mgCOD/ gVSS). The highest yield is for when the sample was supplemented with 200 ppm of trace metals and 100 ppm of vitamins while the lowermost rate is for the test conducted with 500 ppm of trace metals and 200 ppm of vitamins. Increasing the vitamins concentration from 100 to 200 ppm with 500 ppm of biostreme, slightly improved the result. This indicates that 500 ppm of trace metals can be inhibitory to the sludge growth and process, however addition of vitamins in high concentrations of metals, improves the process.

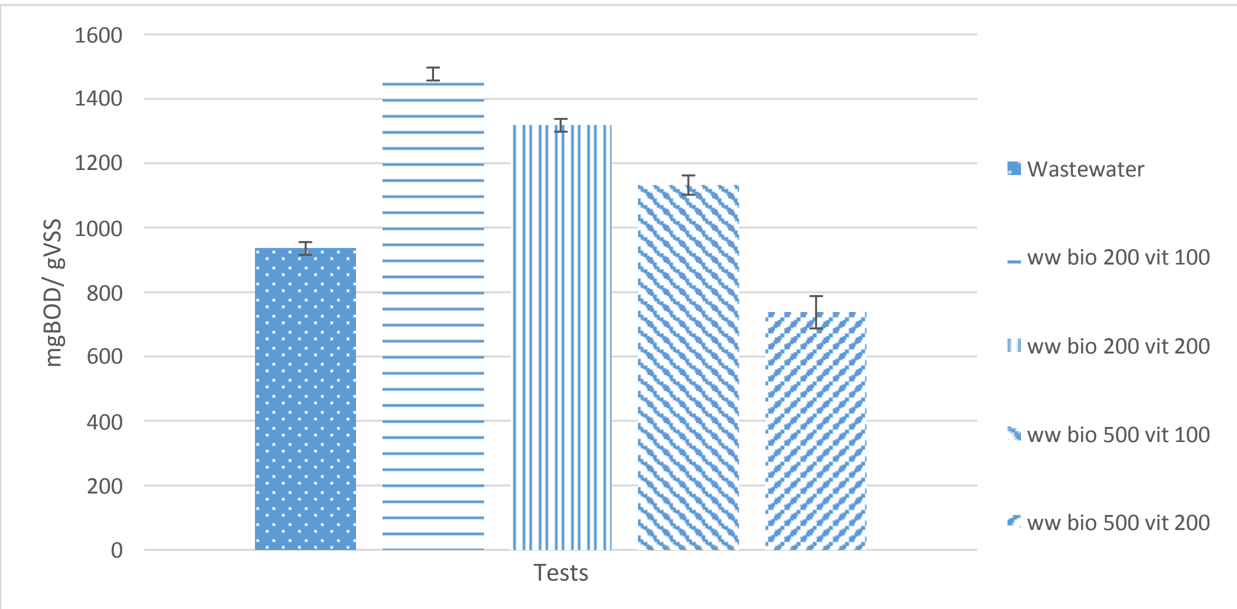


Figure 3- 6: Effect of biostreme in mixture with vitamins solution addition on BOD tests done with RAS as the seed

Compared to the control test, the stimulant phenomena happened in the organic removal process when the BOD bottle was supplemented with 200 ppm of metals and 100 ppm of vitamins. The overall effect of vitamins and trace metals is shown in figure 3-7.

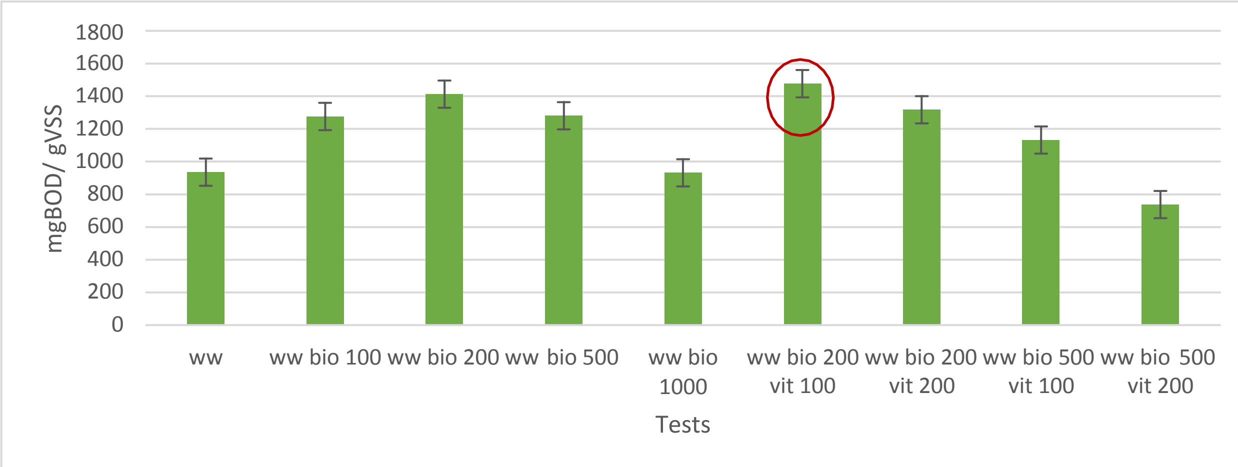


Figure 3- 7: Overall effect of additives on tests done with RAS

Vitamins. With the aim of evaluating the effect of singular vitamins on organic removal and biodegradation of BOR process a specific configuration was applied. Seven individual vitamins were supplemented to the RAS; Biotin, Palmitate, Niacinamide, Pantothenic, Ascorbic, Folic acid. In this specific test a different biostreme which did not contain copper and the vitamin mixture were also examined.

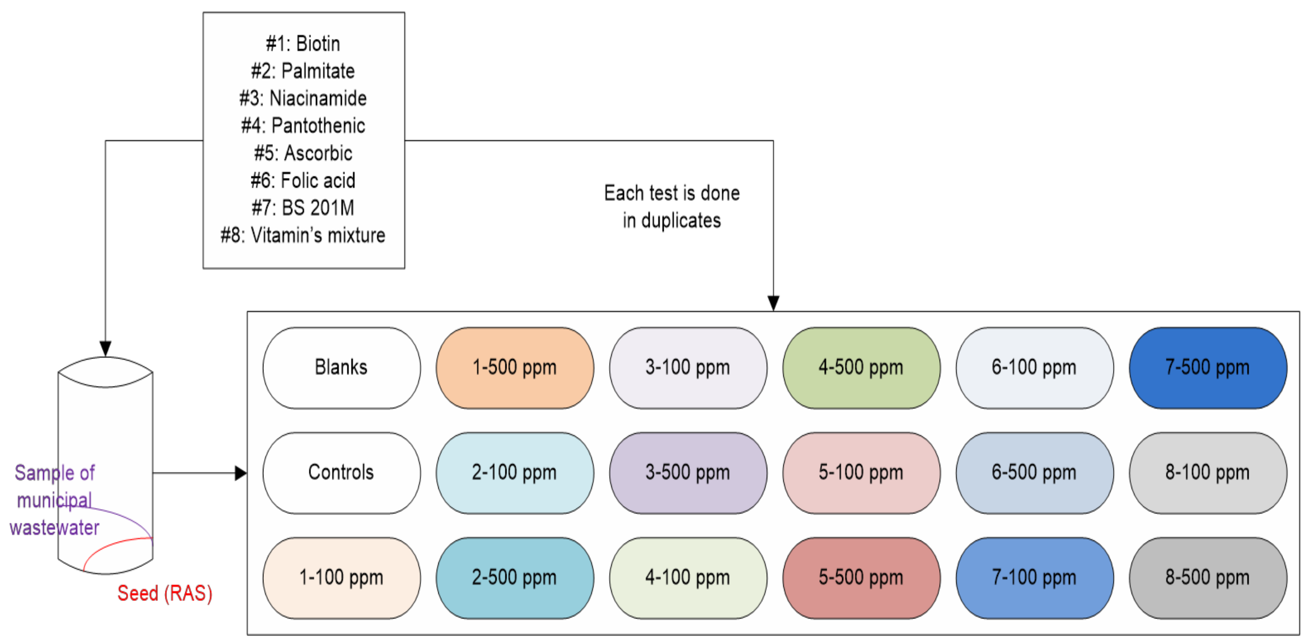


Figure 3- 8: Experimental setup for the test done with vitamins only as the additives

All tests were done in duplicates to minimize the errors. Like previous tests all test bottles contained three main components; seed (RAS), sample (municipal wastewater) and aerated water furthermore, as indicated in figure 3-9, two concentrations of each additive was studied (100 and 500 ppm)

WW: Wastewater with no additives

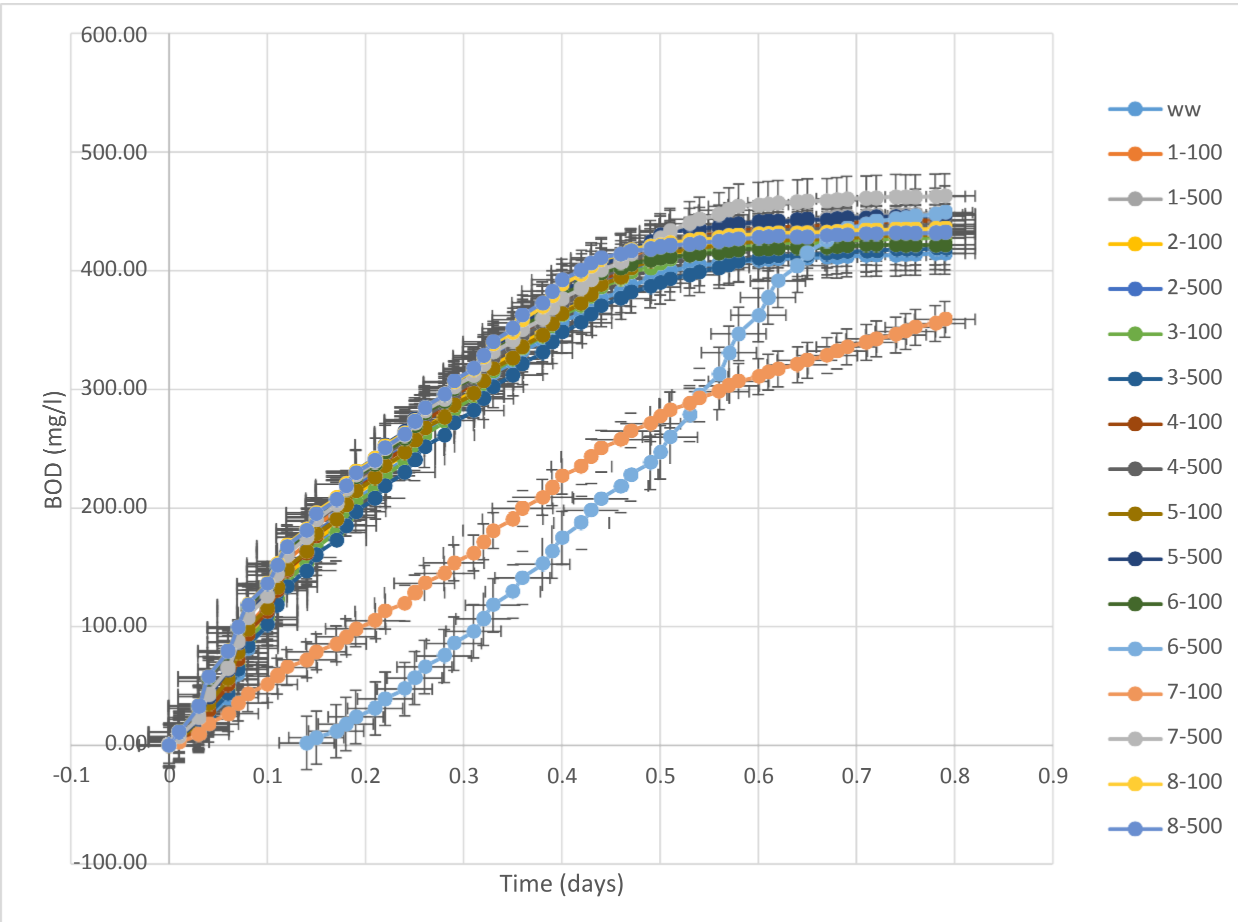


Figure 3- 9: Overall effect of vitamins addition on tests done with RAS as seed

The initial BOD results show a similar trend in almost all test bottles, however the test supplemented with 100 of the biostreme and 500 ppm of folic acid had a different behavior comparing to other tests.

Based on the organic removal rates, the maximum proportion (approximately 30% higher) of organic removal KgBOD/m^3.day, was for the test implemented with 500 ppm of folic acid.

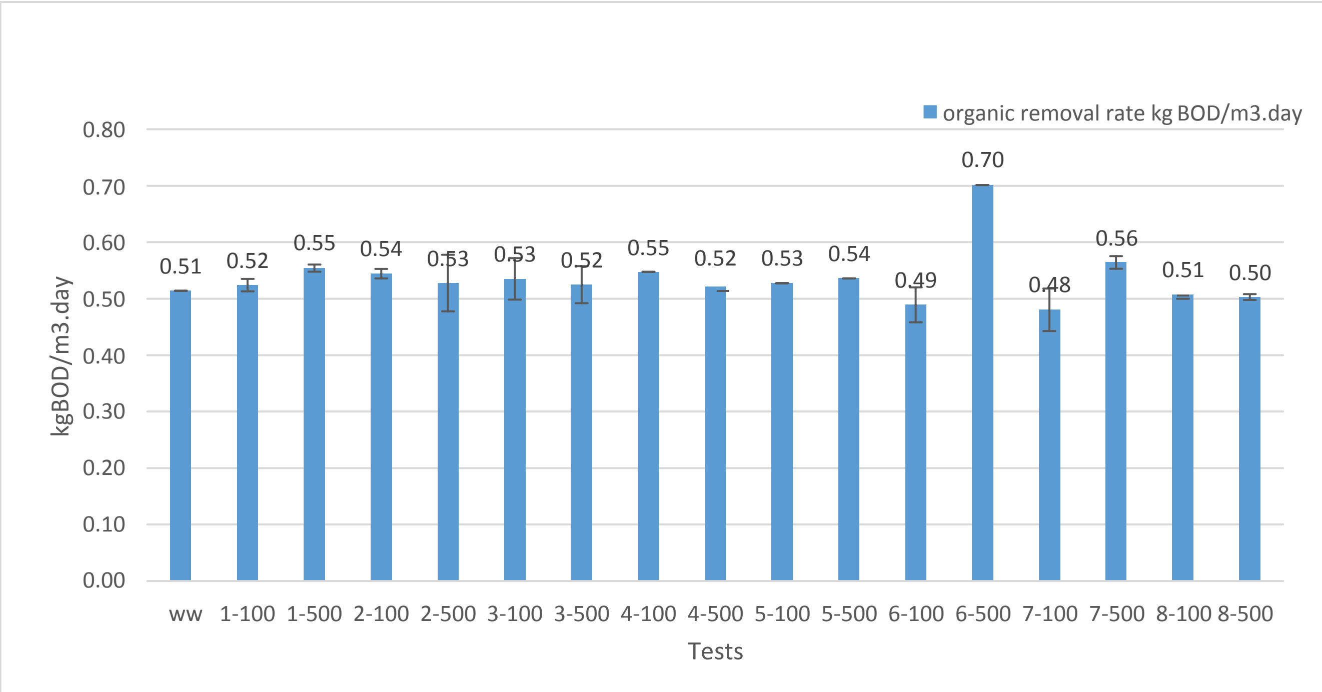


Figure 3- 10: Overall effect of vitamins on organic removals on tests done with RAS as the seed

With the aim of better comparison between the organic removal rates, the results were normalized by the VSS of the final sample taken from each test bottle after five days of operation. (figure 3-10)

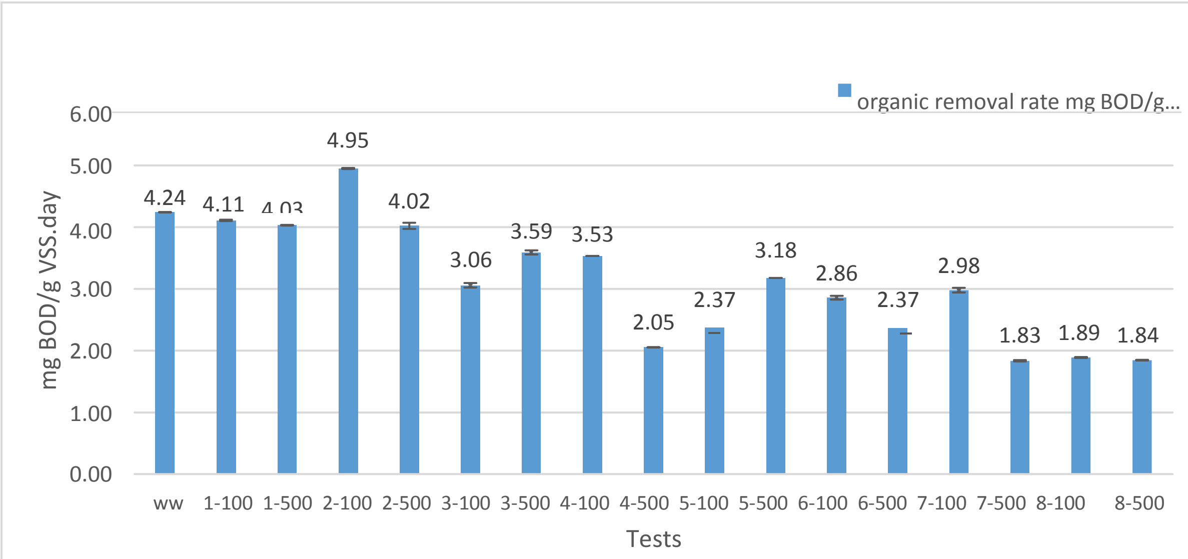


Figure 3- 11: Overall effect of vitamins on organic removals on tests done with RAS as the seed

After normalizing the data by gVSS, the graph shows a completely different trend in which addition of folic acid no longer resulted in the highest rate of organic removal. The highest rate was observed when the municipal wastewater was supplemented with 100 ppm of biotin, this is when addition of other additives had adverse effects on the removal rates comparing to the control test.

3.8. Activated sludge (AS)

Similar arrangement was implemented for AS, collected from a laboratory sized activated sludge system in which the process was fed with municipal wastewater (Humber treatment plant, Toronto, Ontario) and the design factors were formed based on the real-life wastewater treatment plant. The purpose of varying the seed was to evaluate the influences of additives (trace metals and vitamins) on the organic removal process in biodegradability tests.

Figure 3- 12: Activated sludge laboratory experimental setup

The activated sludge system consisted of six separate bioreactors which were connected to feeding bottles, clarifiers and effluent bottles through tubes and pumps. The sludge settled in each clarifier was removed and used as the seed for biodegradability tests. (figure 3-12)

The effect of 100 to 500 ppm of trace metals and 100 to 200 ppm of vitamins was studied over the test. Addition of 500 ppm of the biostreme resulted in a significant increase in the yield. The Organic removal rate was approximately doubled with 500 ppm of the trace metals compared to the control systems.

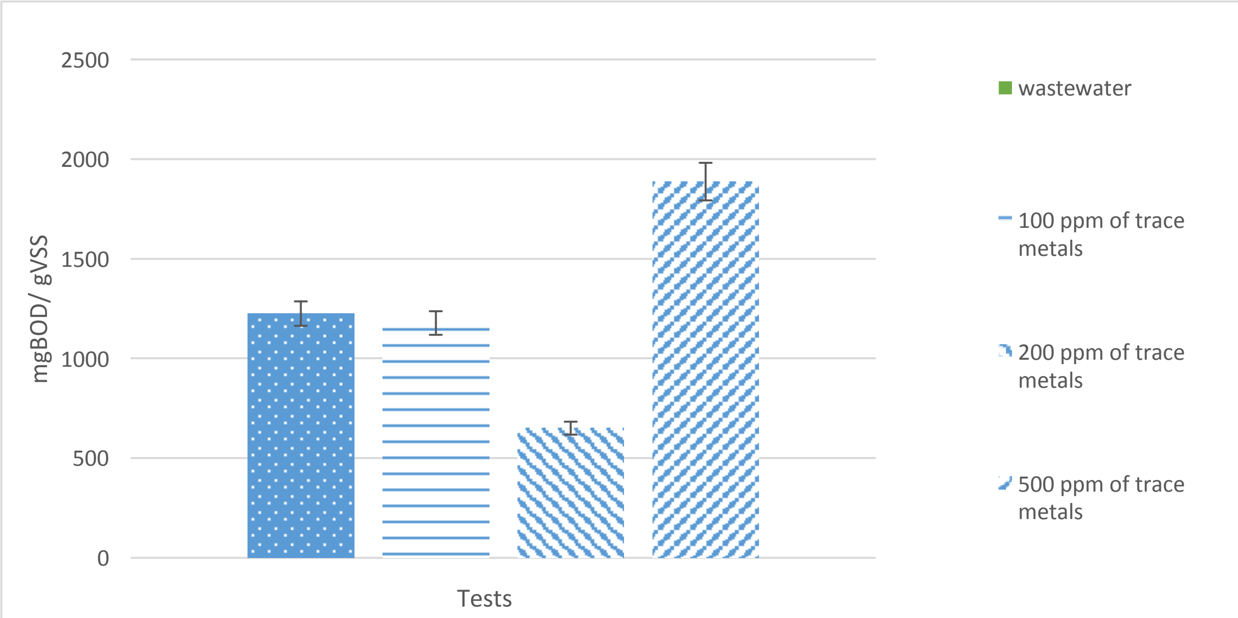


Figure 3- 13: Effect of biostreme addition to the tests done with AS as the seed

Based on the results, addition of 200 ppm of trace metals had an inhibitory effect on the process since the lowest BOD removal was observed in this test. It can be determined from the AS test that only 500 ppm of biostreme has a stimulation effect on the metabolic rate of the microorganisms.

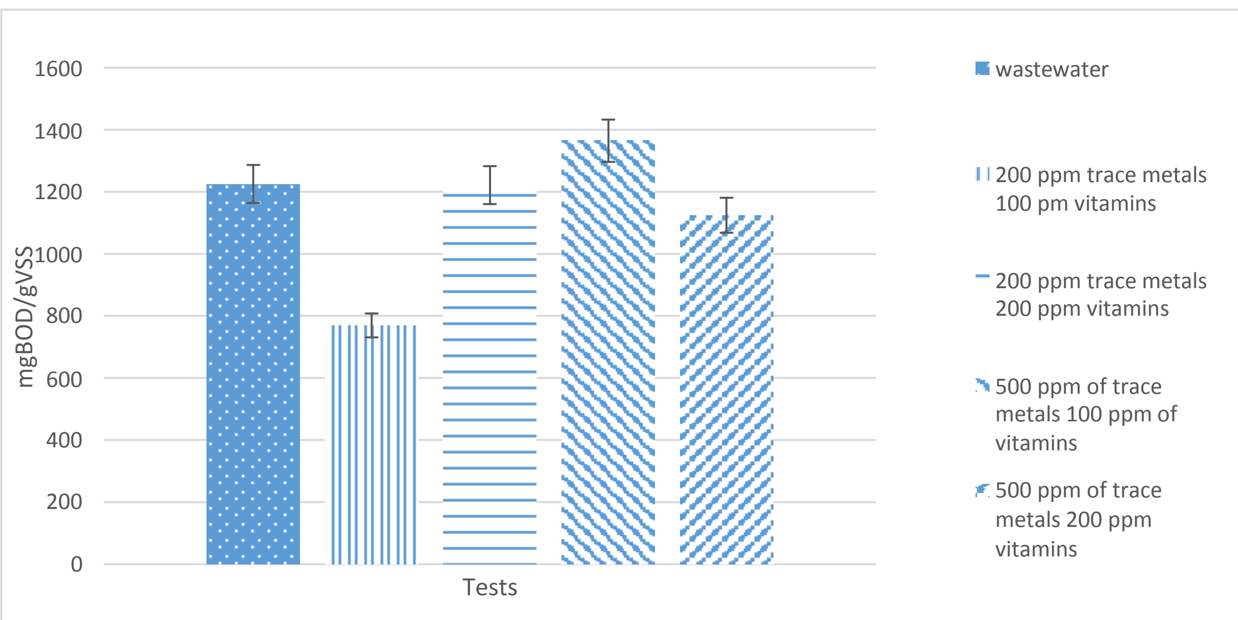


Figure 3- 14: Effect of biostreme and vitamins solution addition to the tests done with AS as the seed

It can be concluded from figure 3-14 that the addition of 200 ppm of the biostreme inhibited the process noticeably, while addition of vitamins depressed the inhibition effect to the level that addition of 200 ppm of vitamin had a very comparable behavior to the test done with 100 ppm of trace metals. In the tests done with combination of metals and vitamins the highest yield of the process is for the experiments with 500 ppm of the biostreme and 100 ppm of the trace metals while increasing the vitamin's concentration to 200 ppm lowered the removal rate.

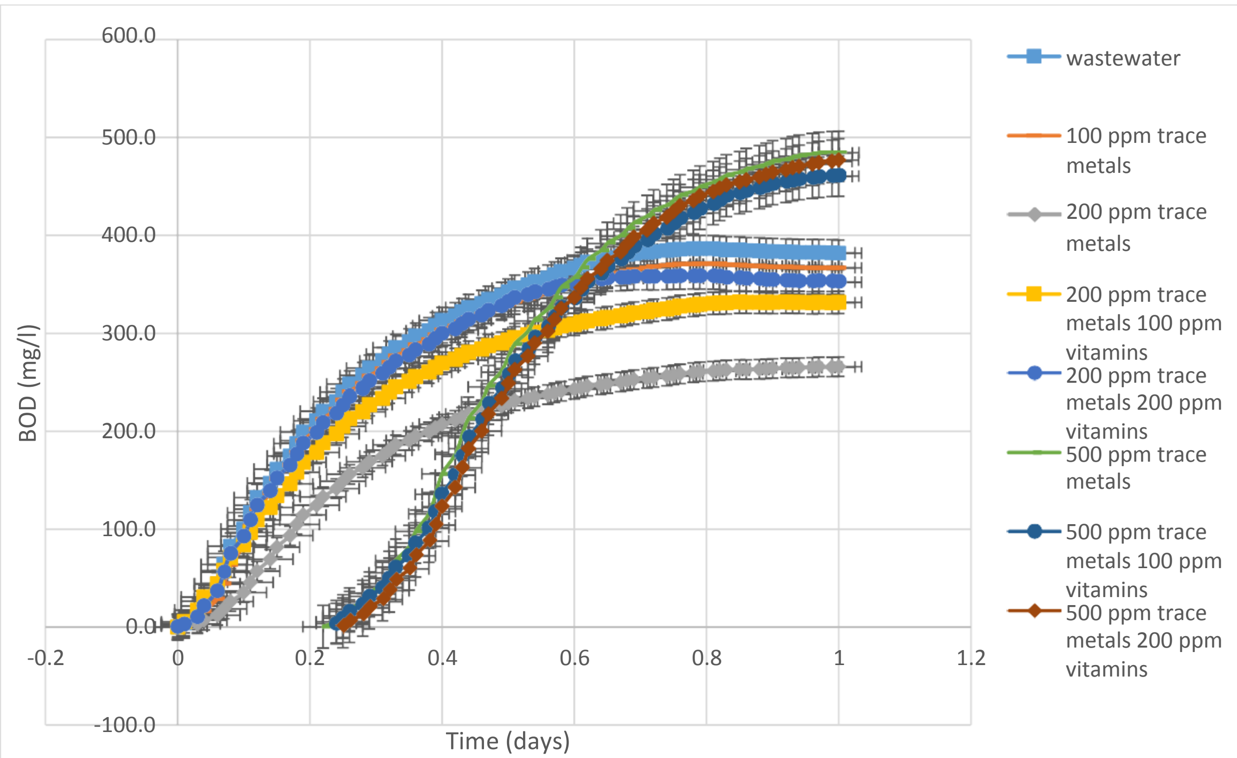


Figure 3- 15: Effect of biostreme and vitamins solution on tests done with AS as the seed

To have a better comparison, the data were normalized with the sample's BOD and VSS. Based on the normalized data (mg BOD/g VSS) the test with 200 ppm of both trace metals and vitamins is almost the same as the control. The least rate was observed with 200 ppm of trace metals added to the organic removal process. There is a considerable stimulation detected by supplementation with 500 ppm of the biostreme.

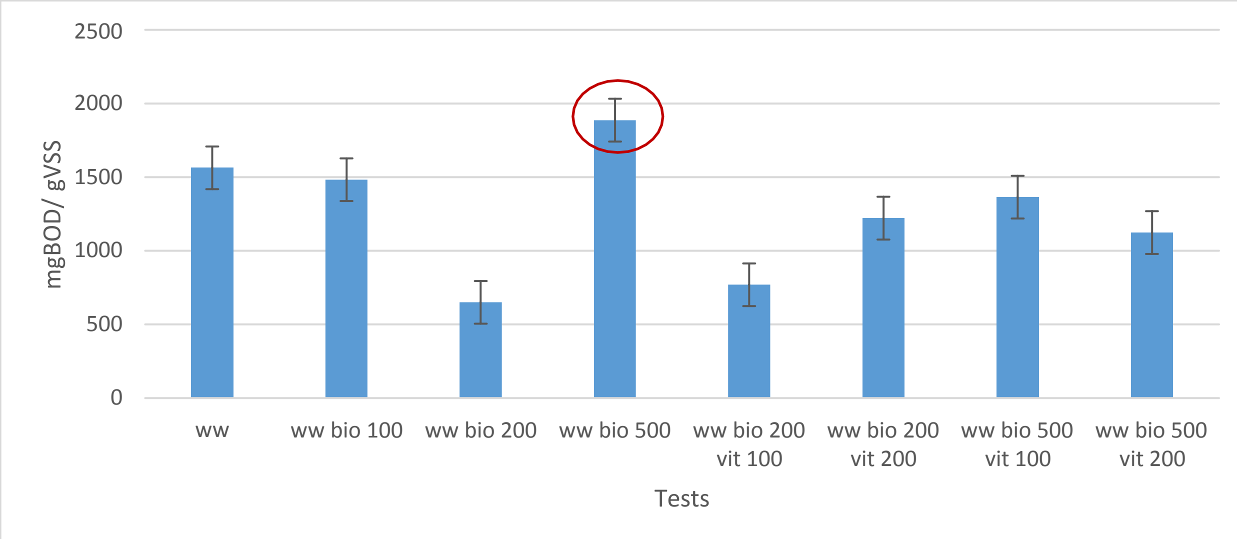


Figure 3- 16: Overall effect of additives (trace metals and vitamins) on tests done with AS as the seed

3.9. Yeast

In a parallel configuration, the effect of trace metals and vitamins were evaluated on biological organic removal from municipal wastewater with yeast as the seed of the process.

This is prepared through addition of a small measured volume of water which contains a sufficient bacterial population, to the BOD test bottles that comprise of both, the aerated water and the sample. This method excludes the difficulty of seed detection that encompasses adequate bacterial populations for oxidization of biodegradable organic matter.

There are specific types of samples which require seed addition due to insufficiency in oxidizing bacteria population in the sewage itself. Performing direct BOD test on such samples is impossible. Determination of the optimum concentration of seed required for the sample is usually done through trial and error method, however it has been discovered that utilization of poly-seed or settled domestic sewage for each sample is usually appropriate.

Similar concentrations of trace metals and vitamins were studied in the test. Concentration of 100, 200 and 500 ppm of the metals mixture was added exclusively to the test bottles whereas, the 100 and 200 ppm of vitamins were supplemented to the test bottles with diverse concentrations of the biostreme.

The effect of various concentrations of additives on the tests with yeast as the seed. All the graphs are very comparable to each other considering the slope and the final concentration of BOD, except the tests completed with 500 ppm of the biostreme. The test with 200 ppm of trace metals seems to have a higher rate of organic removal.

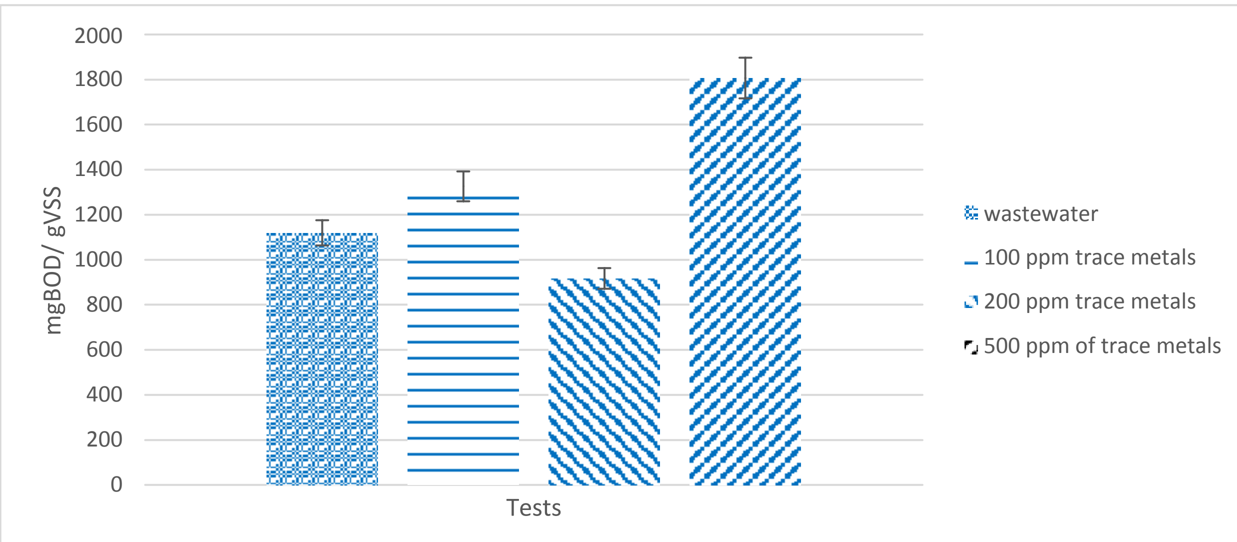


Figure 3- 17: Effect of biostreme addition on tests done with yeast as the seed

For a better evaluation of the results, after excluding the blank in addition to correction by volume and sample (real wastewater) characteristics, figures 3-17 and 18 were established. Based on the figures, the highest rate of organic removal (mgBOD/ gVSS) was for the test with 500 ppm of trace metals. The lowest rate of the organic removal is for the addition of 200 ppm of trace metals and 100 ppm of vitamins, this is when addition of 100 ppm more of the vitamin solution reduced the negative effect of high concentration of metals on the biological process.

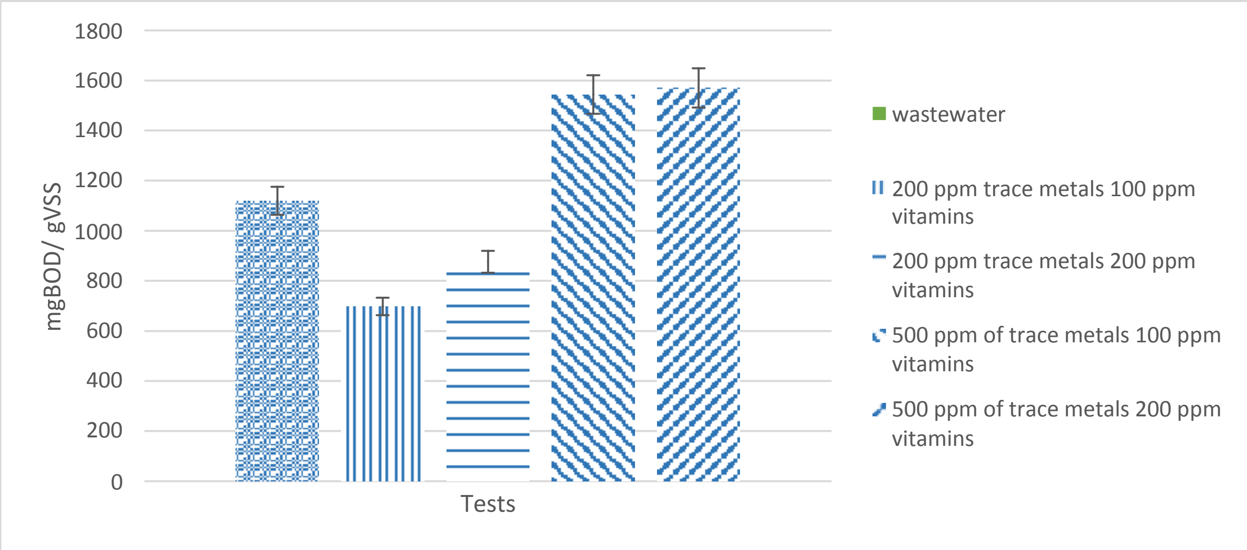


Figure 3- 18: Effect of biostreme and vitamins addition on tests done with yeast as the seed

An overall comparison between all the yeast results show that addition of 500 ppm of the biostreme has the most beneficial effect on the BOD removal process.

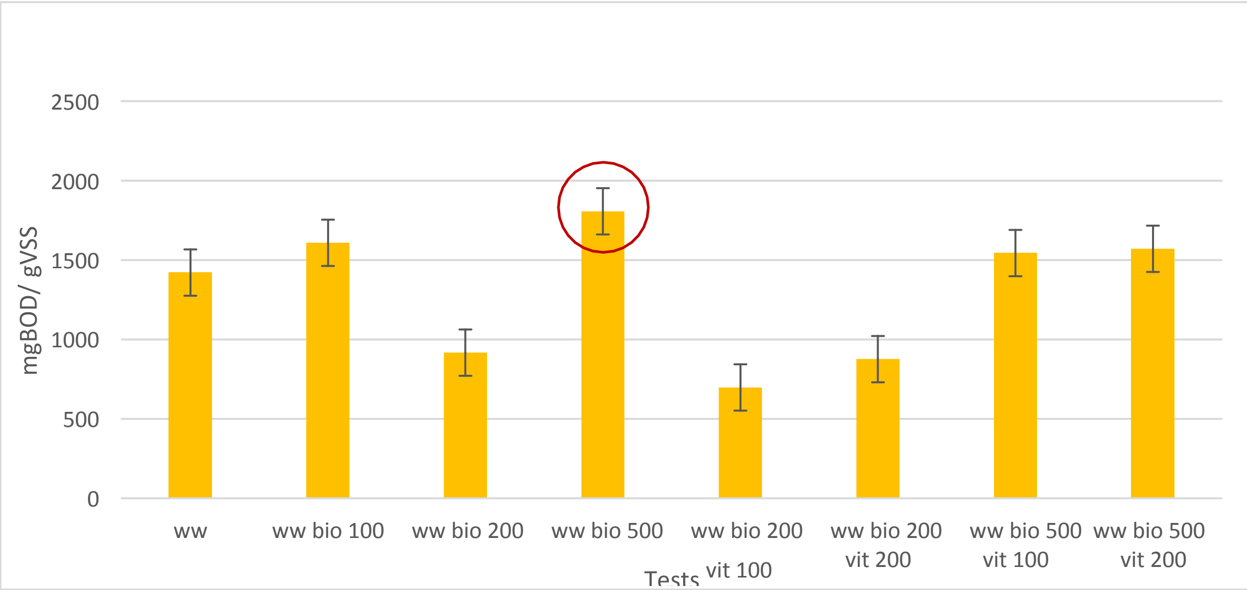


Figure 3- 19: Overall effect of additives (trace metals and vitamins) on tests done with yeast as the seed

3.10. Modeling results

The existing microorganisms in the wastewater stream utilize the available oxygen concentration and oxidize the organic matters present in the waste. The BOD determination is a simple indication of the organic materials present rather than the dissolved oxygen concentration. Complete oxidation stoichiometry can be as shown in the following equation;

$$C_nH_aO_bN_c + (n+a/4-b/2-3c/4)O_2 \rightarrow nCO_2 + (a/2-3c/2)H_2O + cNH_3$$

Equation 5: organic removal process stoichiometry

In general, the oxygen consumption by microorganisms is a first order process in which in a closed system the oxygen consumption rate is calculated by $L= L_0e^{-kt}$ where k is the rate constant and L indicates the oxygen concentration at time t while L_0 is the initial oxygen concentration. To show the oxidation of BOD in the sample $L=L_0-L_0e^{-kt}$ can be used.

The model and experimental results were compared to understand the outcomes from the activated sludge BOD batch tests. There are a few of the comparison graphs from the both the six initial tests and the vitamins test to compare the practical and the simulated data;

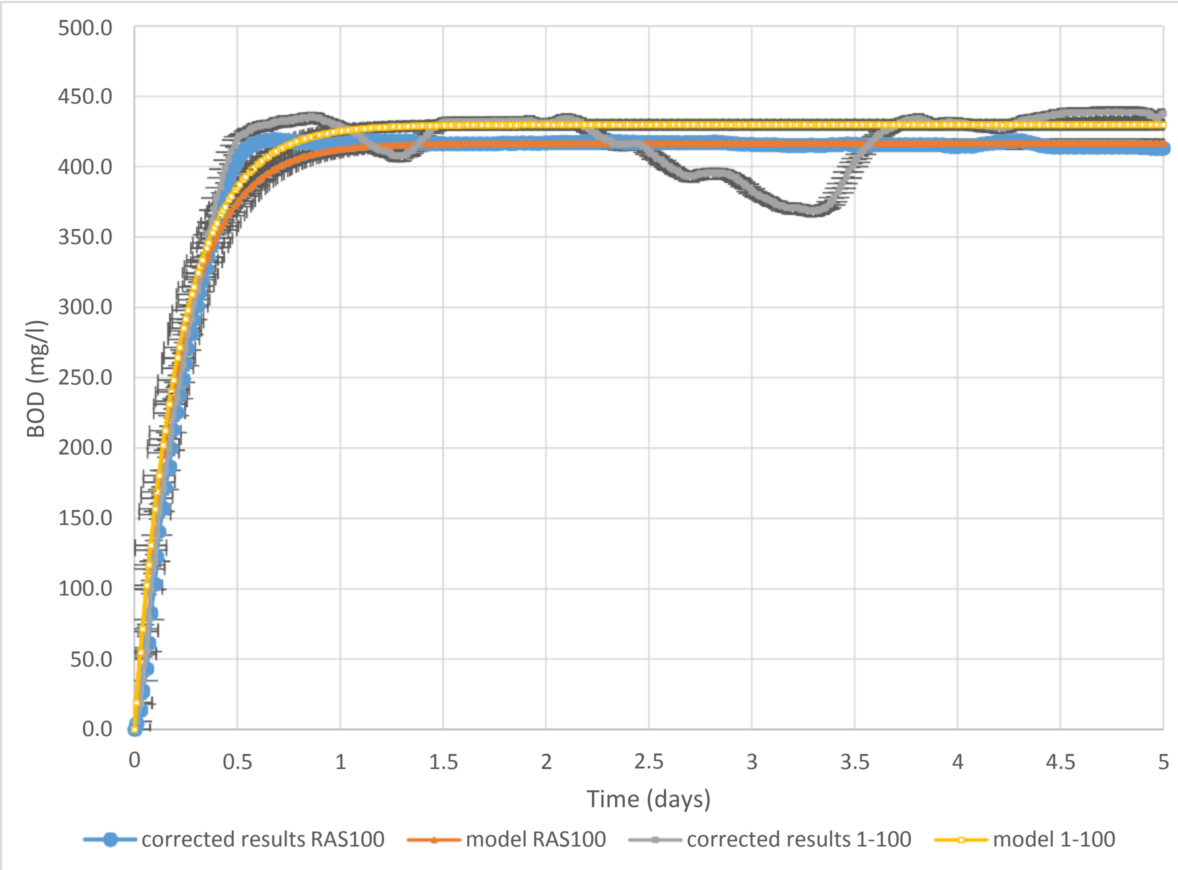


Figure 3- 20: Comparison between the modeled data and the experimental data

The comparison graphs show similar results from both the corrected experimental results (corrected by removing the effect of blank tests) of the BOD tests and the modeled results. Results from test done with 100 ppm of trace metals in RAS is compared with the data from 100 ppm of biotin. As observed, the modeling and actual experimental data are very similar. There are slight fluctuations in vitamin's experimental data, however the average result is a good match to the model. Similar behavior was observed in another test configuration indicated subsequently;

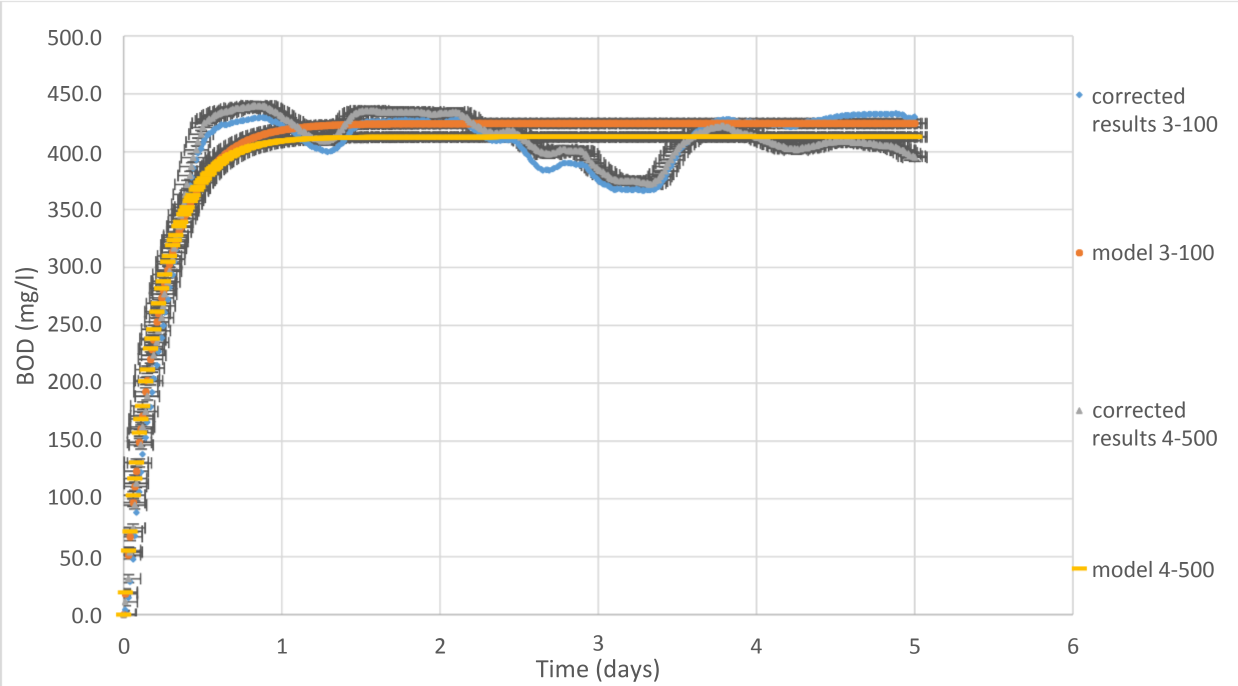


Figure 3- 21: Comparison between the modeled data and the experimental data

3.11. Conclusion

Presence of a trace metal's mixture (biostreme) and vitamin solution in municipal wastewater being treated in an activated sludge process resulted in various effects on the biological organic removal metabolic rate and solid's removal rate. Either the positive or the negative effect depends on the concentrations of the biostreme and vitamin solution.

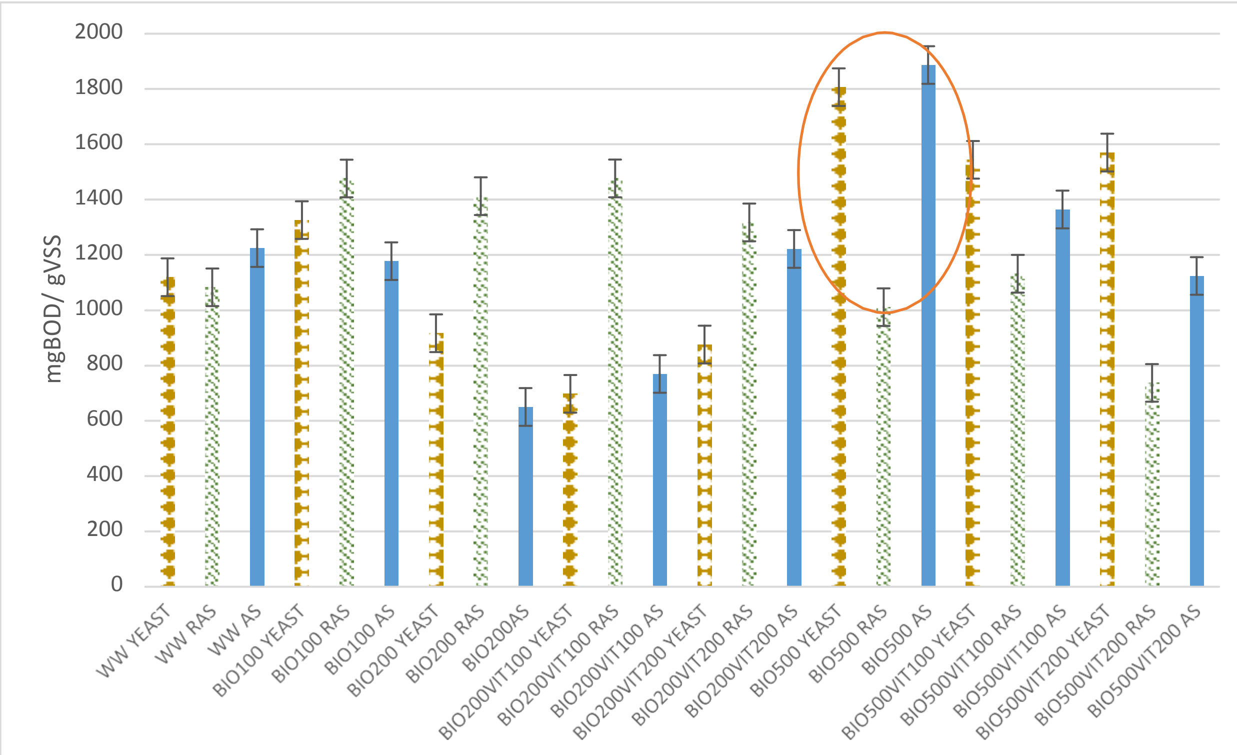


Figure 3- 22: Overall organic removal rates with both different additives and seeds

Different configurations demonstrate that in average, addition of 500 ppm of the biostreme had the maximum rate of organic removal. It can be concluded from the BOR tests that RAS has a slightly different comportment comparing to the AS and the yeast. Supplementation with 200 ppm of the biostreme had the lowermost yield of organic removal amongst all tests.

All the biodegradability tests illustrate a noticeable different behavior when the sample is supplemented with 500ppm of the biostreme both individually or in mixture with the vitamin's solution. The initial results from the BOD graphs indicate a noticeable decrease in the DO (mg/l) to negative BOD concentrations.

This leads to consideration of different environmental factors in the 500-ppm supplementation case, such as food to microorganism's ratio.

Addition of single vitamins resulted in fluctuated BOD removal rates where in some specific cases the removal percentage was higher than the control test.

Chapter 4: Colloids and particulates removal rates in diverse concentrations of biostreme and vitamins solution

4.1. Introduction

All wastewater streams encompass solid and liquid components. The initial objective of all treatment systems is solid deletion from the liquid segment. Solid removal processes vary from physical treatments such as; bar screens, filtrations, clarifiers, centrifuges, sedimentation tanks, etc. to chemical processes. The second stage of treatment focuses on the liquid phase where chemical and biological processes have the main roles (Jarusutthirak & Amy, n.d.).

Mutually the solid and liquid phases contain an assortment of miscellaneous constituents, that are combined physically but not necessarily chemically. The solid phase of the wastewater stream can be divided into three main categories; settleable solids, suspended solids and colloidal solids, from which the liquid phase components are colloids, soluble substances and ions (Jarusutthirak & Amy, n.d.).

Colloids are minor sized particles spread in the solution due to an adsorbed exterior charge. These solids are a combination of homogenous and heterogenous substances, which are undistinguishable to the naked eye and cannot be eliminated by filtration, yet membranes have the potential to remove them from the liquid phase.

There are a number of different forms of solids detected in liquid phase; solutions, solid suspension and colloidal dispersions, while the conformation of these categories is based on the solids particle dimensions (Implications Author, Levine, Tchobanoglous, & Asano, 1985).

Settleable solids are referred to as particles with sizes larger than one micrometer with specific gravity of more than 2.6. colloidal matters are spread due to a surface charge with an average size of between one nanometer to one micrometer. Smaller sized particles (< 1 nm) are categorized in chemical compounds or ions groups.

Larger particles removal is straightforwardly accomplished through physical gravitational separation methods, while colloids create a significant separation problem. Solved particles and ions removal processes necessitate chemical variations in the wastewater solution (Implications Author et al., 1985).

Colloids particles are stabilized in wastewater streams due to existence of negative surface charges. The similar charges cause resistance between the particles and prevents flocs formations and settlement (Jarusutthirak & Amy, n.d.).

Colloids can be categorized into two chief groups; hydrophobic and hydrophilic. The latter is principally formed by hydrated organic molecules in presence of water. The solvated form of these particles is thermodynamically stable, moreover the source of the electrostatic surface charges for these solids is the ionisable groups which have the role of transforming molecules into macro-ions in solutions.

Hydrophobic contain small colloids with no attraction for water. The surface charges attract the other ionic species in the solution and generates a charge layer, surrounding the colloid particle which causes instability for the solids.

Based on recent years' wastewater treatment processes expansions and strict legislations on effluent qualities, there is a growing necessity towards advanced biological wastewater treatment processes (Barjoveanu & Teodosiu, 2010).

There are certain positive features and advantages which prioritize the use of membrane processes over other treatments, however there are number of diverse factors such as technical and economical parameter's assessments that need to be considered in advance (Barjoveanu & Teodosiu, 2010).

Ultrafiltration (membrane process) system design for wastewater recycling with consideration of both the technical and economical features, relies on comprehensive laboratory and pilot scale testing.

Attainment of optimum ultrafiltration process performance, requires a determination of process parameters such as feed flow, membrane pressure, etc. The most important limiting factor is permeating flux decay due to membrane fouling.

After comprehensive pre-treatments, yet most wastewaters hold a large number of solids, organic and inorganic compounds, suspended solids and most outstandingly colloidal substances, in various concentrations which have the crucial role in pore blocking and fouling in the membrane systems (Barjoveanu & Teodosiu, 2010).

Soluble microbial products which display the features of hydrophilic organic colloids, have a noteworthy part in the membrane fouling and flux deterioration in wastewater reuse processes.

Soluble microbial products are principally soluble organics generated from biological wastewater treatment processes, extant in the effluent. The microbial compounds are mainly produced through substrate metabolism, biomass growth and decay. Presence of this category of solids in wastewater effluent not only effects the final COD, BOD and TOC levels, but it also creates constraints to reuse applications where membrane filtration processes are implemented. A major loss of productivity due to membrane fouling is a considerable issue regarding colloids presence in the wastewater's effluent (Jarusutthirak & Amy, n.d.).

As microorganisms are the core factors of organic matter composition, operational factors affecting them have received exclusive consideration in the recent years. A variety of different factors have the potential to alter the growth curve and performance productivity of the microorganisms. In this chapter the effect of micronutrients, trace metals and vitamins, supplementation on biological organic removal process efficiency was studied through specific batch tests. COD removal rates along with the solids removal with a special focus on colloids and particulates were the central concern of this part of the research project.

4.2. Materials and methods

Evaluation of BOR process efficiency and solids removal rates was the main objective of this section of the study. To achieve this goal several batch BOD tests were conducted.

The device utilized for this process was BOD trak II in which six ambient test bottles are employed to assess the oxygen demand concentration over time. The device is fully automated with a working principal of relationship between pressure drop and BOD concentration linked to organic matters in the wastewater solution.

In this arrangement, the BOD is calculated by a physical method without any necessities for chemical procedures. Environmental factors; temperature, diluted wastewater and seed solution composition, 48-hours aerated water, dilution factor and quantities of the nutrient packs were constant in entire tests, not including the additional trace metals and vitamins concentrations. After five days of experimentation, the samples were taken as of individual test bottle and the COD fractions and solids were studied.

Each configuration involved number of test bottles. A precis volume of sample, seed and water were placed in the amber bottles calculated based on the BOD range of the solution and were coupled with the device. All bottles were connected to a pressure sensor which monitored the pressure fluctuations versus time in the head space of the bottle at all time.

A quantity of air was placed in the overhead of the liquid phase in the test bottles which contained 21% oxygen. Microorganisms with a focus on bacteria existing in the sludge sample consumes the dissolved oxygen during the BOD test with the aim of oxidizing the organic matters, which, results in air pressure decline. The pressure drop in the bottle is right connected to the BOD, because it regulates the amount of oxygen used up by the bacteria remaining in the sample. All the bottles were fully agitated continually with a magnetic stirrer, the constant moving resulted in additional oxygen supplies for the sample and more food for the bacteria. The suitable mixing preserves rapid oxygen transmission from liquid phase to the gas phase. Each device consists of six, 492 ml isolated bottles. The depth and height of each bottle is 10.3 and 3.9 inches. Method used for the tests was standard in which, sample, seed and dilution water added to the bottles were individually 110 ml, 10-35 ml and 350 ml, in respect. The purpose behind demonstrating volumes for each parameter is the BOD range stated in the device instructions. The expected BOD range for this experiment was in between 0 to 350 mg/l. All the tests were automatically ended after 5 days of operation.

The experimental configurations were arranged in three different partitions. The first subdivision was with the utilization of municipal wastewater (Humber treatment plant, Toronto, Ontario) as the sample, and RAS, (Humber wastewater treatment plant, Toronto, Ontario) by means of the seed in the experiment. The sample was the municipal wastewater in both other tests, however the seed differed to AS collected from bottom part of a laboratory clarifier in activated sludge system and yeast.

The AS was obtained from a research laboratory activated sludge system, which comprised of six aeration bioreactors, six clarifiers and twelve effluent and influent tanks. The system was fed day-to-day and continuously with municipal. (figure 4-1)

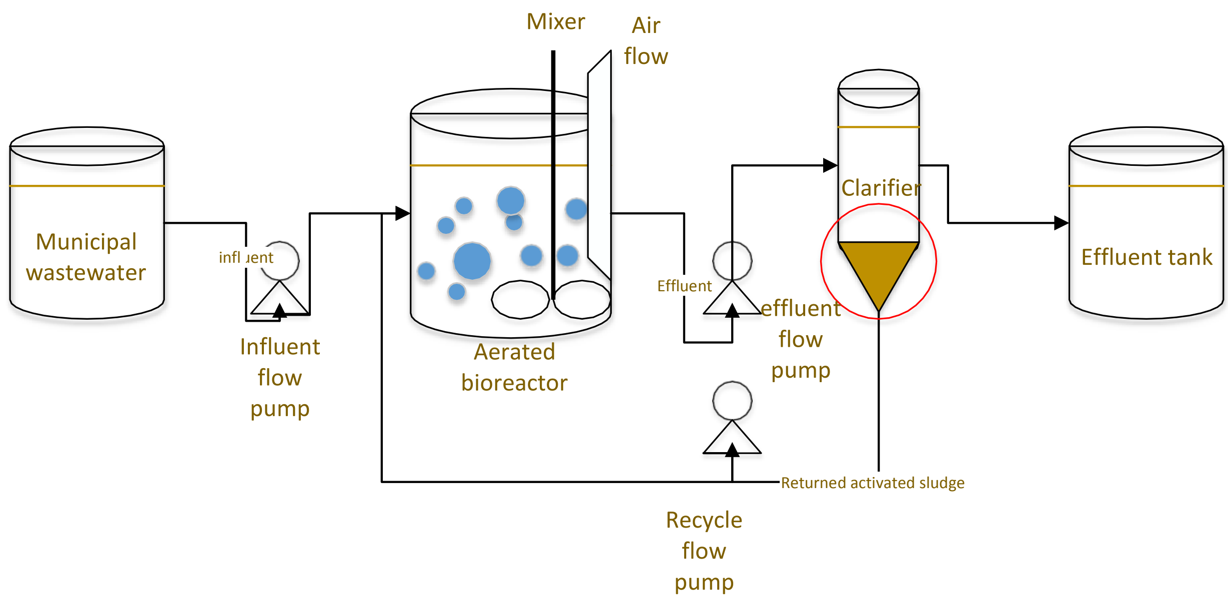


Figure 4- 1: Activated sludge system

The aerated distilled water for the batch BOD test was made two days prior to each test. A combination of distilled water and predetermined amount of nutrients were aerated with air tubes for a 48 hours. The samples and seeds were brought to 19 to 21 ˚C before each test. The wastewater used in the experiment as the sample was wholly standardized with no large stable or buoyant solids. specific quantities of sample, seed and aerated water were added to the bottle. Two potassium hydroxide pellets were added to the seal cup on top of the bottle.

Various concentrations of vitamins and trace metals were added to the batch bottles. Each conformation was completed in trios, with the purpose of discounting errors or any out of range numbers.

Table 4-1: Different configurations of BOD tests

Study / seed	Biostreme ppm/l	biostreme ppm/l	biostreme+vitamins ppm/l	biostreme+vitamins ppm/l
1 / RAS	100	200	200 + 100	200 +200
2 / AS	100	200	200 + 100	200 + 200
3 / yeast	100	200	200 + 100	200 + 200
4 / RAS	100	500	500 + 100	500 + 200
5 / AS	100	500	500 + 100	500 + 200
6 / yeast	100	500	500 + 100	500 + 200
7 /RAS	0	0	0 + 100	0 + 500

The municipal wastewater's (sample), total and soluble COD and VSS were measured before the test's initial startup. Both COD and VSS determinations were prepared on unfiltered samples with standard methods for wastewater analysis. All the data were automatically collected and imported in to spread-sheets. The dilution factor in these specific experiments was 1.45.

Each sample collected from the test bottles was analyzed in the COD content. The Total COD (tCOD), Soluble COD (sCOD) and Semi Soluble COD (ssCOD) were measured in each sample moreover, the VSS and the TSS were measured with the aim of data normalization.

4.3. Results and discussion

Activated sludge. There are no considerable alterations in sCOD removal between control tests and the bottles supplemented with concentrations of the micronutrients. However, the ssCOD decreased by 64% and 76% with addition of 100 and 500 ppm of the biostreme, respectively.

Addition of vitamins to the 500 ppm biostreme did not result in higher colloidal removal rate. The colloidal concentration dropped from 91 mg/l to 19 and 6 mg/l with addition of 100 and 500 ppm of the biostreme, respectively.

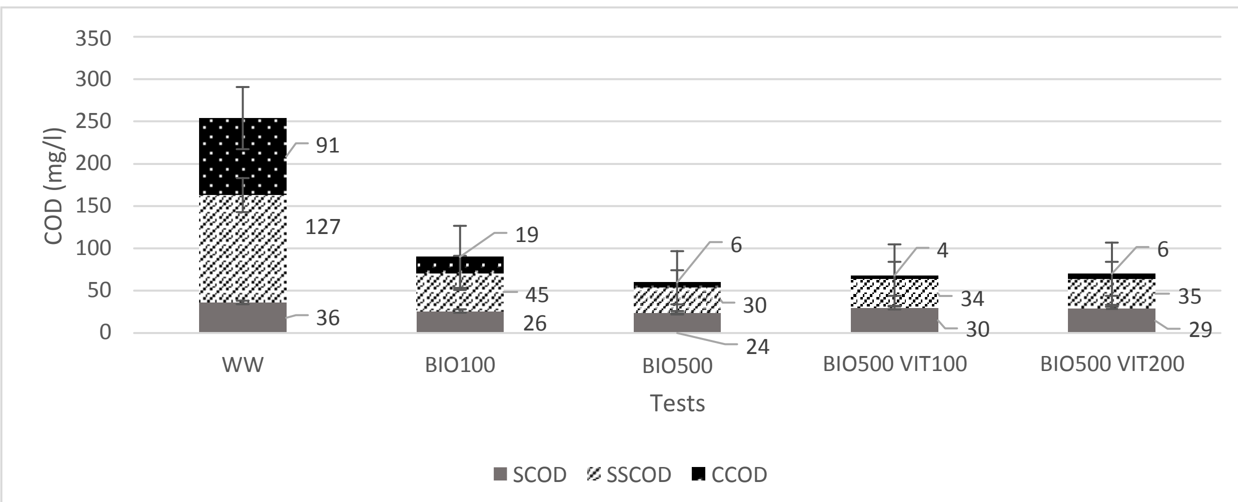


Figure 4- 2: Biodegradability analysis in tests done with AS as the seed

The following chart shows the results for the particulate and colloidal matter in different tests compared to the control bottle.

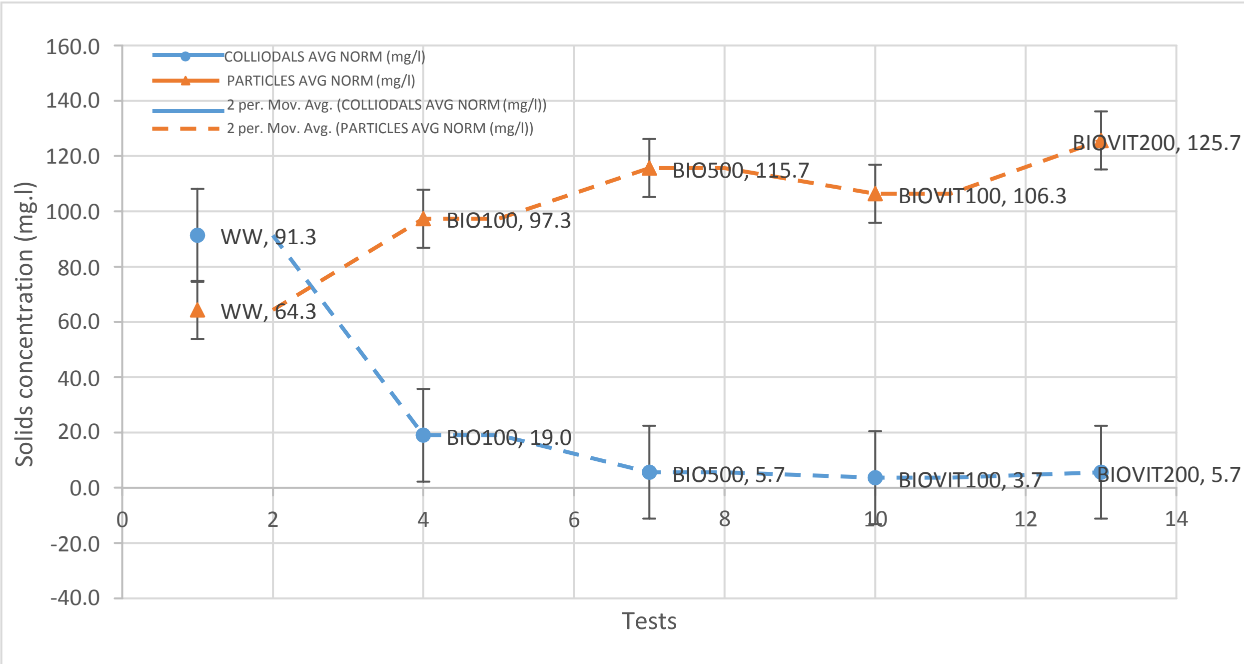


Figure 4- 3: Colloids and particulates concentration alteration by addition of biostreme and vitamins solution in tests done with AS as the seed

Yeast. The samples were analyzed after five days of organic removal process. tCOD, sCOD and ssCOD as well as total and volatile suspended solids were measured in each sample individually.

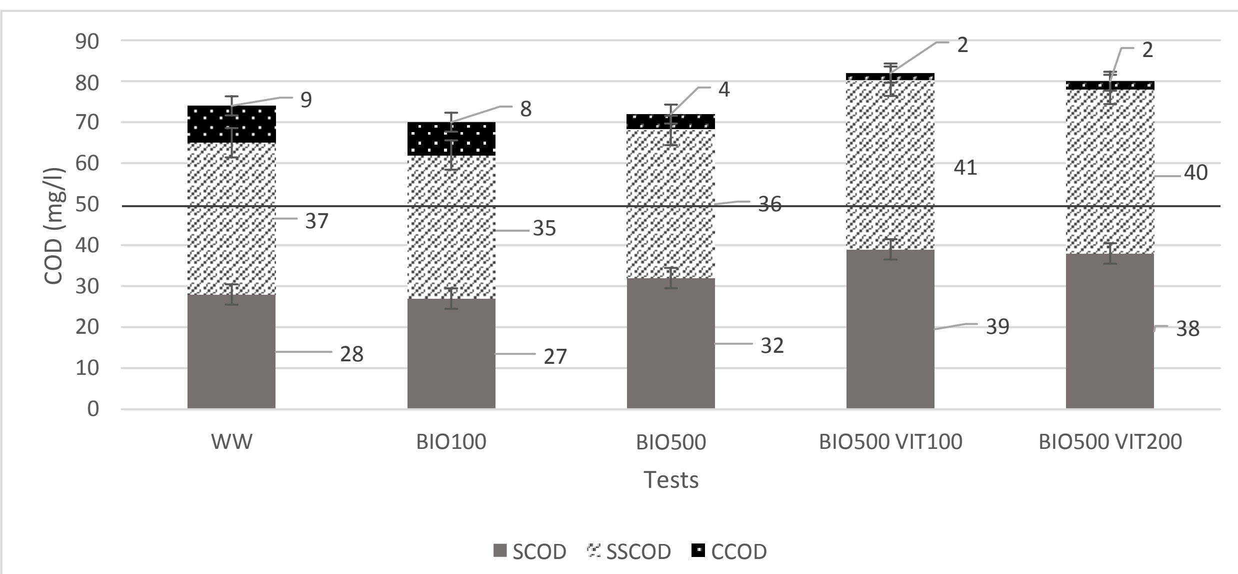


Figure 4- 4: Biodegradability analysis in tests done with yeast as the seed

Results from this test show a similar scheme comparing to the test done with AS as the seed. The colloids concentration dropped from 9 mg/l to 4 and 2 mg/l with addition of 500 ppm of biostreme and 500 ppm of biostreme plus 100 ppm of vitamins, respectively.

The declining slope of the colloidal matter's concentrations is less than the previous test since the initial concentrations are lower due to addition of yeast as the seed instead of the activated sludge.

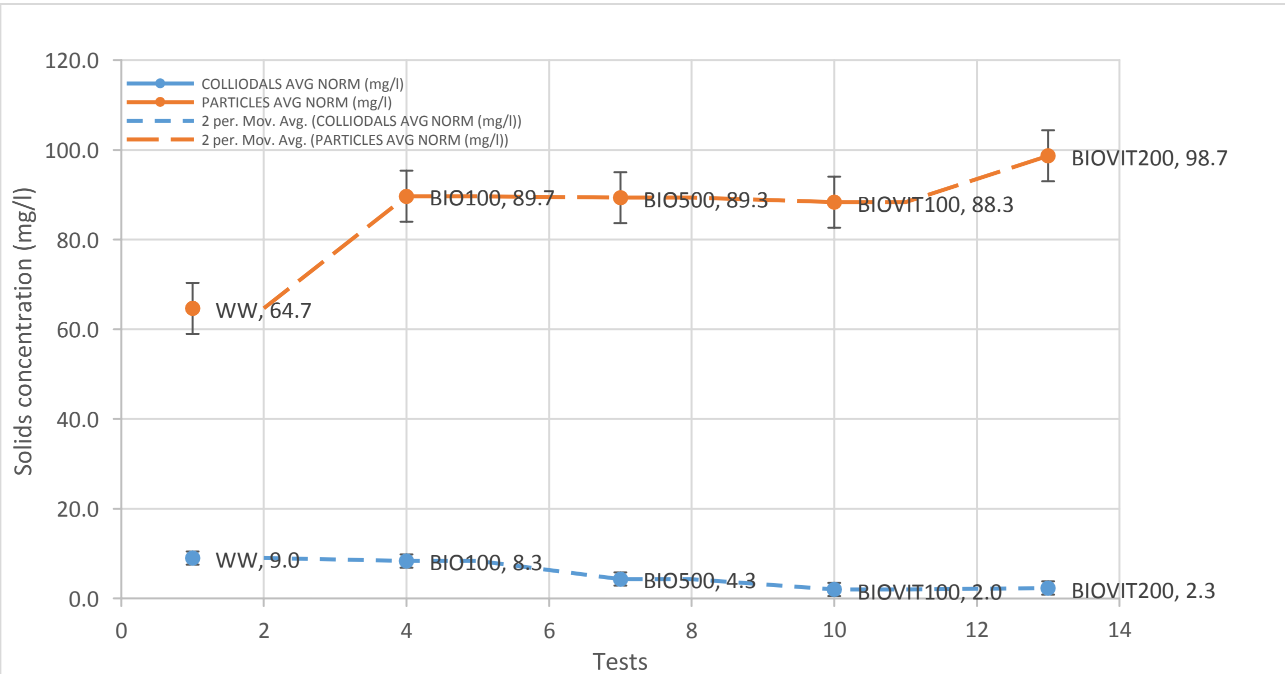


Figure 4- 5: Colloids and particulates concentration alteration by addition of biostreme and vitamins solution in tests done with yeast as the seed

Recycled activated sludge. The batch tests with municipal recycled activated sludge resulted in a slightly different scheme of colloidal matter's alteration. The analysis shows a significant drop (approximately 80%) in colloid's concentration when the wastewater was supplemented with 100 ppm of the biostreme, even though addition of 500 ppm of the trace metal's mixture had a completely different influence on the process. The colloid's concentrations augmented with adding higher concentrations of the biostreme and vitamin.

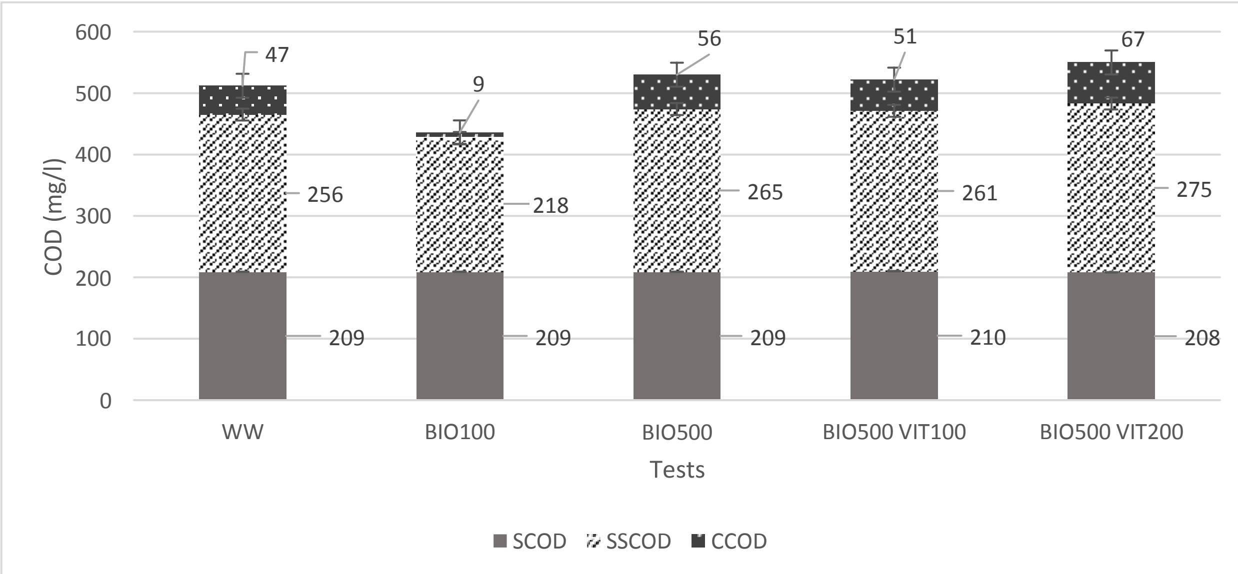


Figure 4- 6: Biodegradability analysis in tests done with RAS as the seed

The colloid's concentration, decreased from 47 to 9 mg/l when the wastewater was supplemented with 100 ppm of the biostreme, whereas this concentration increased to 56 when the biostreme's concentration was increased by 400 ppm. Addition of 200 ppm of the vitamin solution to 500 ppm of the biostreme increased the colloid concentration to 67 mg/l. (figure 4-6)

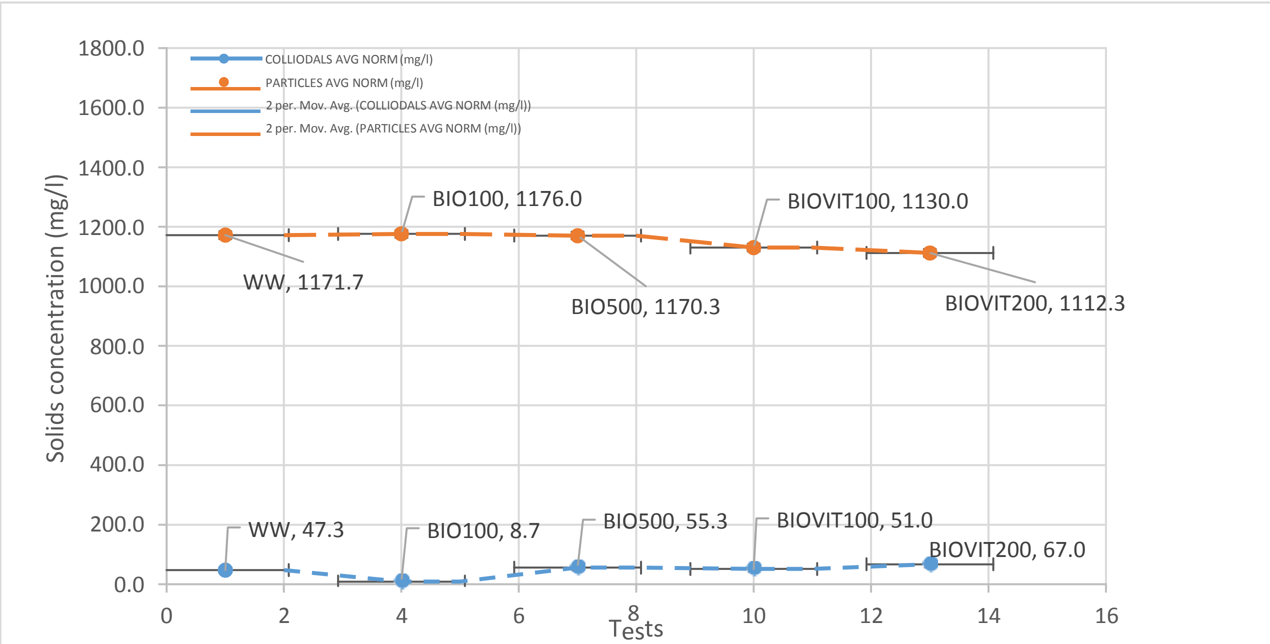


Figure 4- 7: Colloids and particulates concentration alteration by addition of biostreme and vitamins solution in tests done with RAS as the seed

Using the same seed, a similar configuration was tested with supplementation of individual vitamins and vitamin's mixture to evaluate the effect of vitamins addition on the colloids removal rate. Seven individual vitamins were supplemented to the RAS; Biotin, Palmitate, Niacinamide, Pantothenic, Ascorbic, Folic acid. In this specific test a different biostreme which did not contain copper and the vitamin mixture were also examined.

After five days of BOD removal process in Trak II, samples were collected from each test bottle and the CODs and solids were analyzed.

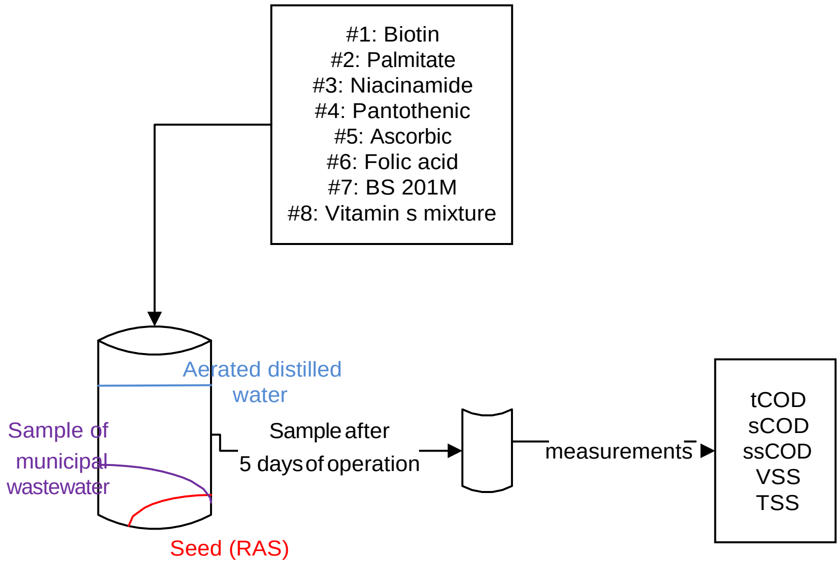


Figure 4- 8: BOD batch tests system setup and analysis for vitamin's addition evaluation

The results show a significant fluctuation in the colloids concentration depending on the vitamin and the concentration added. The lowest colloids concentration was from the test supplemented with 100 ppm of biotin where the concentration was approximately 95% lower than the control test, however increasing the concentration to 500 ppm had a detrimental effect on the removal rate.

Addition of 100 ppm of folic acid not only did not decreased the colloids concentration but the results were 78% higher than the control test, although increasing the vitamin concentration by 400 ppm considerably improved the colloids removal rates. (figure 4-9)

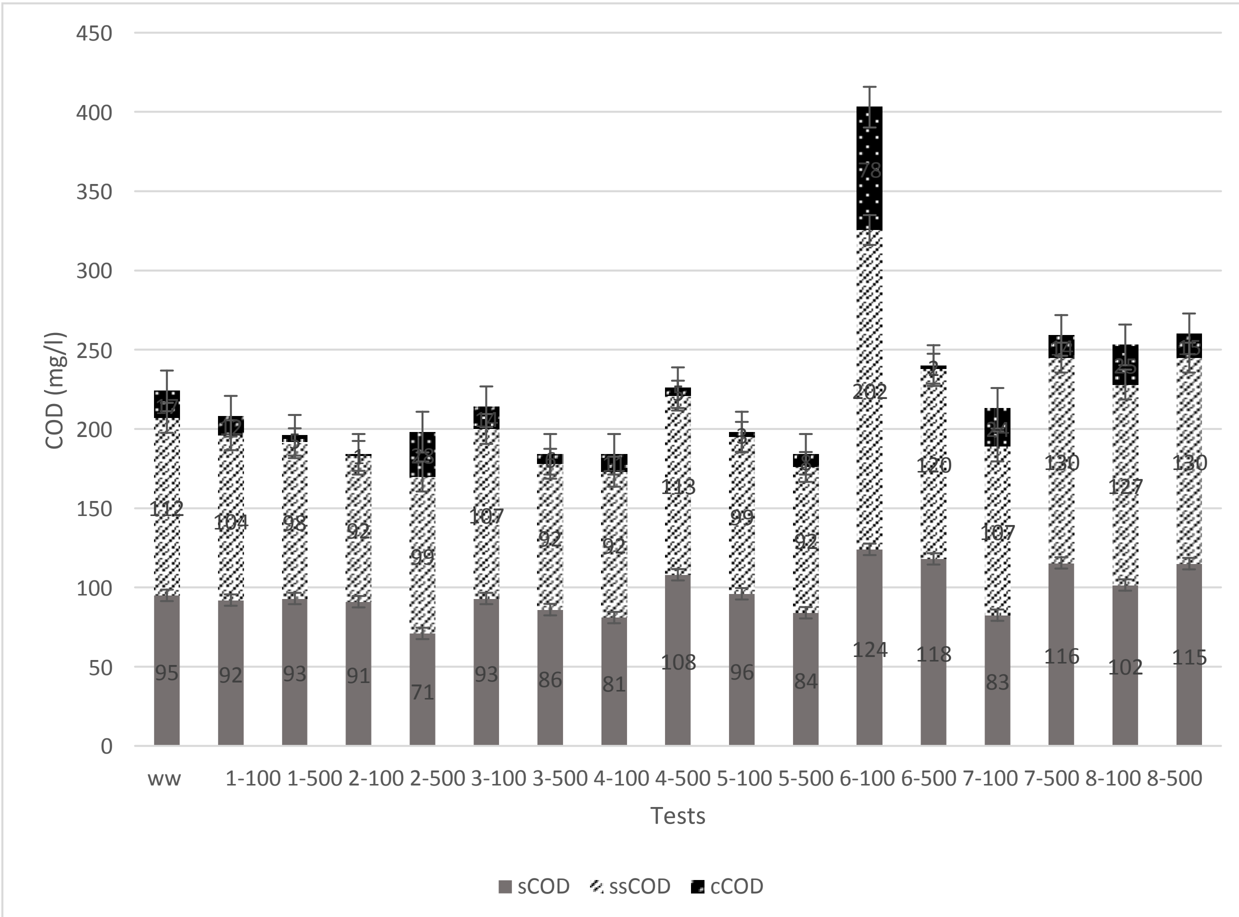


Figure 4- 9: Biodegradability analysis in tests done with RAS as the seed and addition of vitamins

4.4. Conclusion

Results from assorted tests indicate that addition of trace metals and vitamins can beneficially affect the colloids and particulates removal process. Both tests done with AS and yeast as their seed show a positive response to addition of 100 and 500 ppm of the biostreme as well as mixture of the biostreme and vitamin’s solution. In the case where RAS was used as the seed the process only showed a constructive response to addition of 100 ppm of the biostreme.

Different individual vitamins have varied effects on the solids removal rates, where 100 ppm of palmitate can significantly decrease the colloids concentration while 100 ppm of folic acid is extremely detrimental to the process.

Chapter 5: Effect of trace metals and vitamins addition on biodegradability in BOR process in diverse organic loading rates

5.1. Background

In an activated sludge system, organics are degraded through an aeration process in which bacterial growth is promoted. In the process of secondary treatment, the cell acclimation results in significant organic material biodegradation and substantial decrease in BOD content of the influent. This system mainly consists of two factors; the aeration basin in which the cells (X) interact with the substrate (S) and the clarifier where the cells are removed from the water (Nazaroff & Alavarez, n.d.).

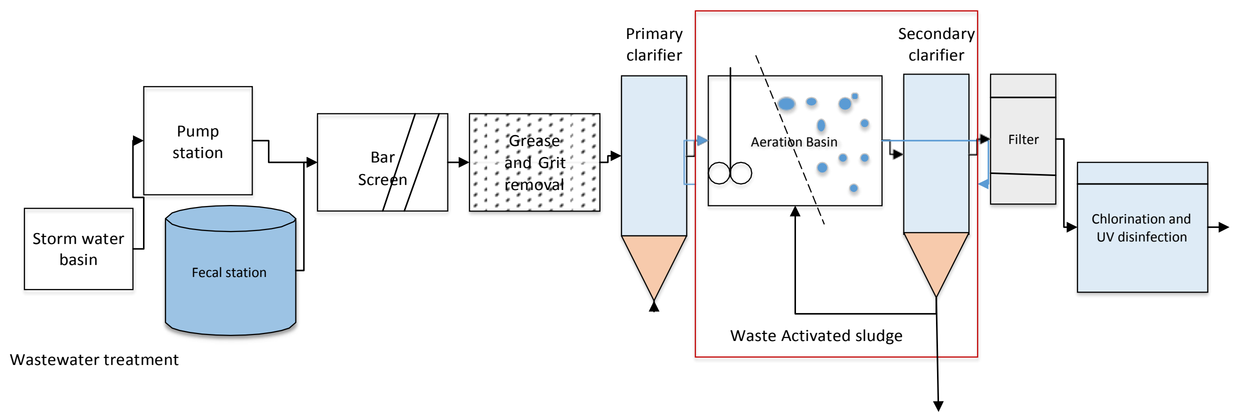


Figure 5- 1: Activated sludge process

There are a variety of organisms that grow and decay through the process based on the substrate composition and flow. There are two fundamental parameters; the substrate and the microorganism's concentrations involved in the treatment system, where S is directly linked to the amount of BOD available for the bacterial cells (Nazaroff & Alavarez, n.d.).

The cell growth is proportional to the substrate consumption rate; $r_g = Y.r_s$ where the equation's coefficient, referred to as yield is approximately 0.6 due to CO_2 emissions from cells. With the aim of cell growth promotion, a fraction of the sludge settled in the bottom of the clarifier is recycled back to the aeration tank. The recycle ratio in a conventional activated sludge system is between 0.25 to 1.

It is important to mention the alterations in substrate concentration because of cell consumption;

$(1/\Theta)(S_{in} – S)= (k_mSX)/(K_s + S)$

Equation 6: Substrate concentration based on cell consumption

In which K_m is mg BOD_5 per mg VSS per day and K_s indicates the mg BOD_5 per liter. Θ is the mean cell residence time.

To fully understand the activated sludge mechanism, the subsequent equation shows the mass balance between the existing, dying and growing cells in the activated sludge system;

$((1 + R)/\Theta + K_d)X= Y(K_mSX/(K_s + S)) +X_u(R/\Theta)$

Equation 7: Mass balance in activated sludge system for biomass

One of the considerable characteristics of the activated sludge system is the F/M ratio defined as the BOD supplied divided by the cells in the aerators;

$F/M= (Q_{in}S_{in})/VX= S_{in}/\Theta X= S_{in}/(S_{in}-S)((1+K_dSRT)/YSRT))$

Equation 8: F:M ratio definition

A significant design parameter in activated sludge process is the F/M ratio. This factor defines the balance between the substrate consumption and biomass generation. F/M ratio is responsible for organic matter decomposition, moreover there are different categories of activated sludge systems based on the F/M ratio in which the conventional method has a ratio of between 0.2 to 0.5.

Increase in F/M ratio results in higher biomass growth and respiration rate, and an increased rate of bacterial BOD removal rate (Nazaroff & Alavarez, n.d.).

Advancement and preservation of a proper, dynamic and diversified microbial population in activated sludge system is the key pathway to effective biological treatment. Organic waste is denoted as food source for the microbial population, besides microorganisms utilize the organic matter to synthesize new cell material and attain energy for maintenance and progression, consequently biological growth encompasses mutually cell synthesis and biodegradation through the bacterial population extant in the biomass (Spencer & Sc, n.d.).

Heterotrophic microorganisms necessitate a multifarious source of organics for growth, whereas the specie obtain the essential energy for growth and maintenance by biodegradation of organic food stream. Aerobic heterotrophs are accountable for major organic matter biodegradation in wastewater treatment.

There are diverse variables that impact the growth and degradation of the organics; pH, temperature, toxicity, nutrient supplementation, F/M ratio, sludge age, etc.

One of the most important features for growth and biodegradation is F:M ratio. Lower F/M ratios result in higher extents of substrate degradation for energy supply and lower degrees of biological solids accretions in the system (Spencer & Sc, n.d.) (Tay, Pan, He, & Tay, 2004).

In conditions with low F/M ratio the absence of metabolism’s resource results in low microbial metabolisms, on the other hand extreme F/M ratios initiate metabolic imbalance (Spencer & Sc, n.d.).

5.2. Materials and methods

The objective; evaluation of the effect of additives (biostreme and vitamin's solution) and organic loading rate (F/M ratio) on biodegradability, resulted in explicit BOD batch tests with three dissimilar F/M ratios and concentrations of biostreme individually and in a combination with the vitamin's solution.

The additives utilized were specific complex mixture of trace metals referred to as biostreme and a convoluted assortment of primarily group B vitamins concentrations. 500 ppm of the biostreme individually and in a combination with 100 ppm of the vitamins were added to test bottles.

Table 5-1: Biostreme composition

Format	**formula**
Cobalt Sulphate	$CoSO_4.H_2O$
Copper sulphate	$CuSO_4.5H_2O$
Ferrous sulphate, heptahydrate	$FeSO_4.7H_2O$
potassium chloride	KCl
Manganese Sulphate	$MnSO_4.H_2O$
Molybdic acid	MoO_3
Nickel (II) Chloride	$NiCl_2.6H_2O$
Zinc sulphate	$ZnSO_4.1H_2O$
Water	H_2O
sulphuric acid	H_2SO_4
Selenius acid	H_2SeO_3
Sodium Tungstate	$Na_2WO_4.2H_2O$

Vanadyl Sulphate	$VO.SO_4.5H_2O$
Yucca shidigera	-
Yeast	-
Gelatin	-
Boron	$Na_2B_8O_{13}.4H_2O$
Niacin amide	B_3
Pantothenic	B_5
D-biotin	B_7
Folic acid	Group of B
Palmitate	Group of A
Ascorbic acid	Group of C

Device used for this test was BOD trak II in which the BOD is measured through a physical method deprived of any chemical measurements. Environmental variables; temperature, wastewater composition and source, aerated water nutritional buffers, aeration method and dilution factor were constant in all tests, exclusive of additional trace metals and vitamins concentrations and the F/M ratio which was linked to seed concentrations.

Each bottle consisted of three main components; seed, sample and aerated water (figure 5-2). As stated in previous chapter the sample of all the batch tests were municipal wastewater taken from Humber treatment plant, Toronto, Ontario. The seed used for this biodegradability test was RAS from the same source as the sample, while the final component was 48-hour aerated distilled water supplemented with specific concentrations of nutritional buffers.

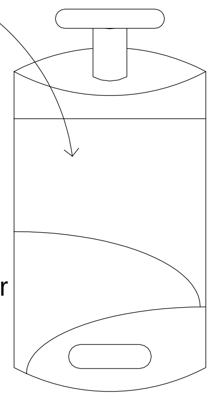


Figure 5- 2: BOD test bottle configuration

Three F/M ratios were applied to the experiments; 0.22, 0.15 and 0.07 mgBOD/gTSS which consisted of 10, 15 and 30 ml of seed in the test bottle respectively. All analysis contained 110 ml of municipal wastewater and a specific volume of aerated distilled water to attain the dilution factor.

Blank and control tests were done in singular bottles without repetition while all supplemented samples were tested in triplicates.

Entire tests were completed after 5 days moreover; the data were gathered as BOD variations against time. The numbers of the organic removals and BOD fluctuations versus time were automatically saved and put in to graphs for the period of the tests. The data were accessible throughout the test and could be merely studied over the BOD assessment. After five days of experiment, the samples were taken from each test bottle for more detailed measurements and analysis.

The setup consisted of 24 separate test bottles, three main components were added and the pressure sensors were placed on top of the bottle's lid. BOD alterations are directly linked to pressure drop in the head space, since it indicates the bacterial oxygen consumption from the remaining sludge in the solution. (figure 5-3)

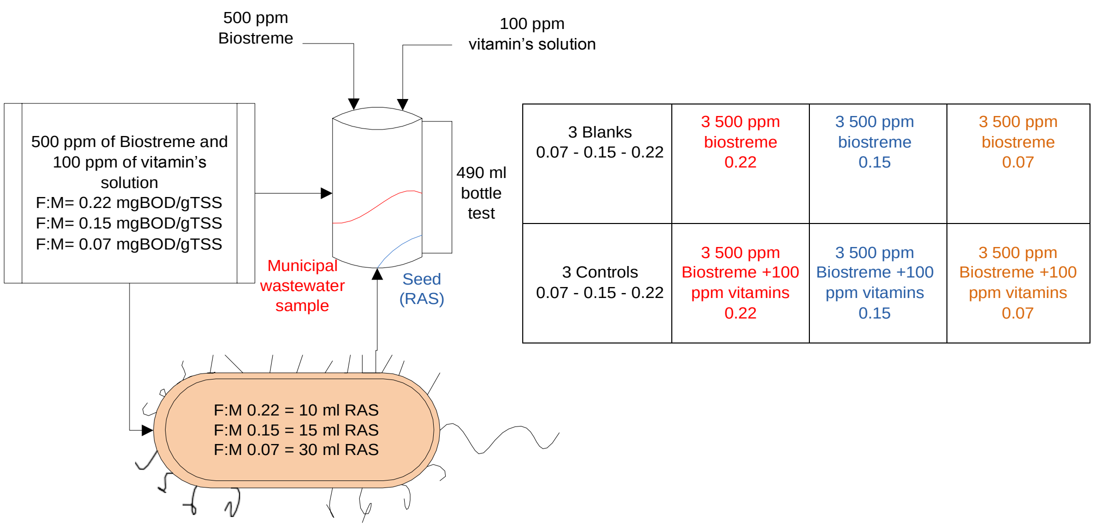


Figure 5- 3: Experimental setup with different organic loadings and additives concentrations

All bottles were completely mixed with magnetic stirrers which maintained suitable oxygen transfer from liquid phase to gas phase.

Various concentrations of vitamins and trace metals were added to the batch system. Each arrangement was done in trios, with the purpose of discounting errors elsewise any out of range data. In each test set, blank tests were employed, to eliminate the dilution water's impact, from the results.

Biochemical oxygen demand can be defined as a parameter stated in mg/l or ppm which regulates the amount of oxygen that the bacteria uptake from the water throughout organic matter oxidization. The BOD measurement is one of the basic determinations of water contamination gradation. For the seeded samples, BOD of the seed must be measured by the blank test bottles advance to other configurations. The BOD in such experiments is required to be corrected as following:

$$BOD = \frac{(BOD\ of\ bottle) - (f\ of\ seed \times BOD\ of\ seed)}{f\ of\ sample}$$

Equation 9: BOD correction equation

After data correction, graphs indicating the alterations of BOD versus time were conducted and compared to understand the impact of the biostreme and vitamins solution in diverse organic loadings on organic removal and biodegradability.

5.3. Results and discussion

Experimental results

Different behaviors were observed based on the system's alterations in both the F/M ratio and additives concentrations. Analysis of 500 ppm biostreme independently and in an amalgamation with 100 ppm vitamins solution was completed in three main F/M ratios, the important results are included in the following.

The initial results from the BOD tests show a similar trend in all configurations, nevertheless the slopes were significantly different, indicating the effect of the shifting parameters.

As it is shown in the graph the overall increasing trend is similar in all different tests, although the initial slope is slightly different when the system is supplemented with additives and the F/M ratio is changed. Based on BOD test results all the systems had an increasing trend of BOD against time up to approximately 14.5 hours and afterwards the BOD is stabled at a constant rate.

Incapability to compare different configurations based on figure 5-4, led us to compare the tests based on their organic removal rates.

The organic removal rates (kgBOD/m^3.day) were calculated for all experimental bottles. With the aim of detailed and exact comparisons between the tests the BOD removal rates were normalized by the solutions VSS content (mgBOD/gVSS).

Comparisons between the tests indicate that, in the control bottles addition of more seed to the test, or in other word reducing the F/M ratio decreased the organic removal rate. The removal rate declined from 0.63 kgBOD/m^3.day to 0.5 by lessening the F/M ratio from 0.22 to 0.07 mgBOD/gTSS in the control tests. Similar trends were observed in the supplemented tests where the organic removal rates with addition of 500 ppm biostreme were 0.65, 0.58 and 0.52 in 0.22, 0.15 and 0.07 F/M ratios in respect. (figure 5-4)

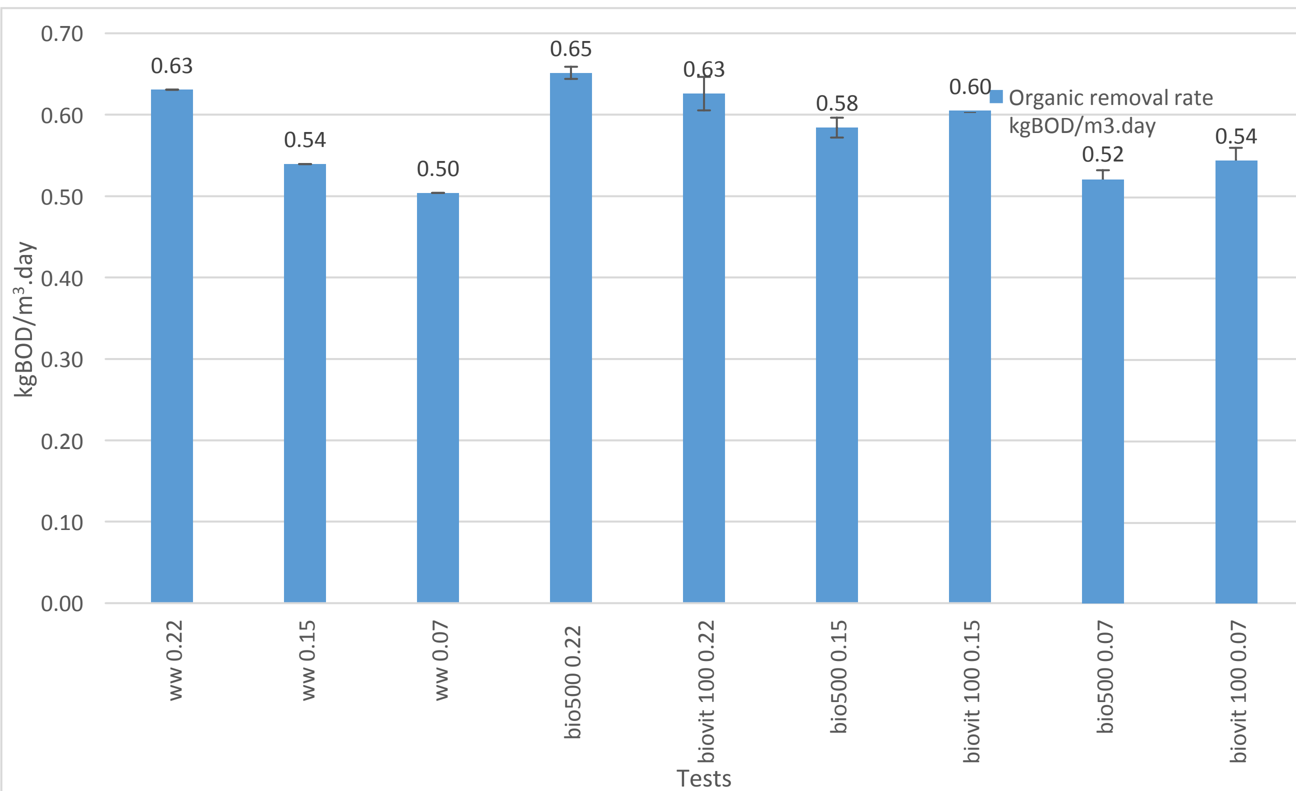


Figure 5- 4: Effect of Biostreme and vitamin addition in different organic loading rates on organic removal rates

The results also show an increasing trend when additives are injected to the system, where addition of 500 ppm of biostreme slightly improved the organic removal rate, however addition of vitamins solution to the 500 ppm biostreme reduced the organic removal rate by 3%.

With the aim of better comparisons between the tests, the organic removal rates were normalized by the VSS of the samples taken from the test bottles after five days of operation.

The results from figure 5-5 indicate a similar trend, where addition of more RAS to the test bottles decreased the organic removal rate. Changing the seed volume from 10 to 30 ml or in other words decreasing the F/M ratio from 0.22 to 0.07 caused a 78% decline in control removal rates.

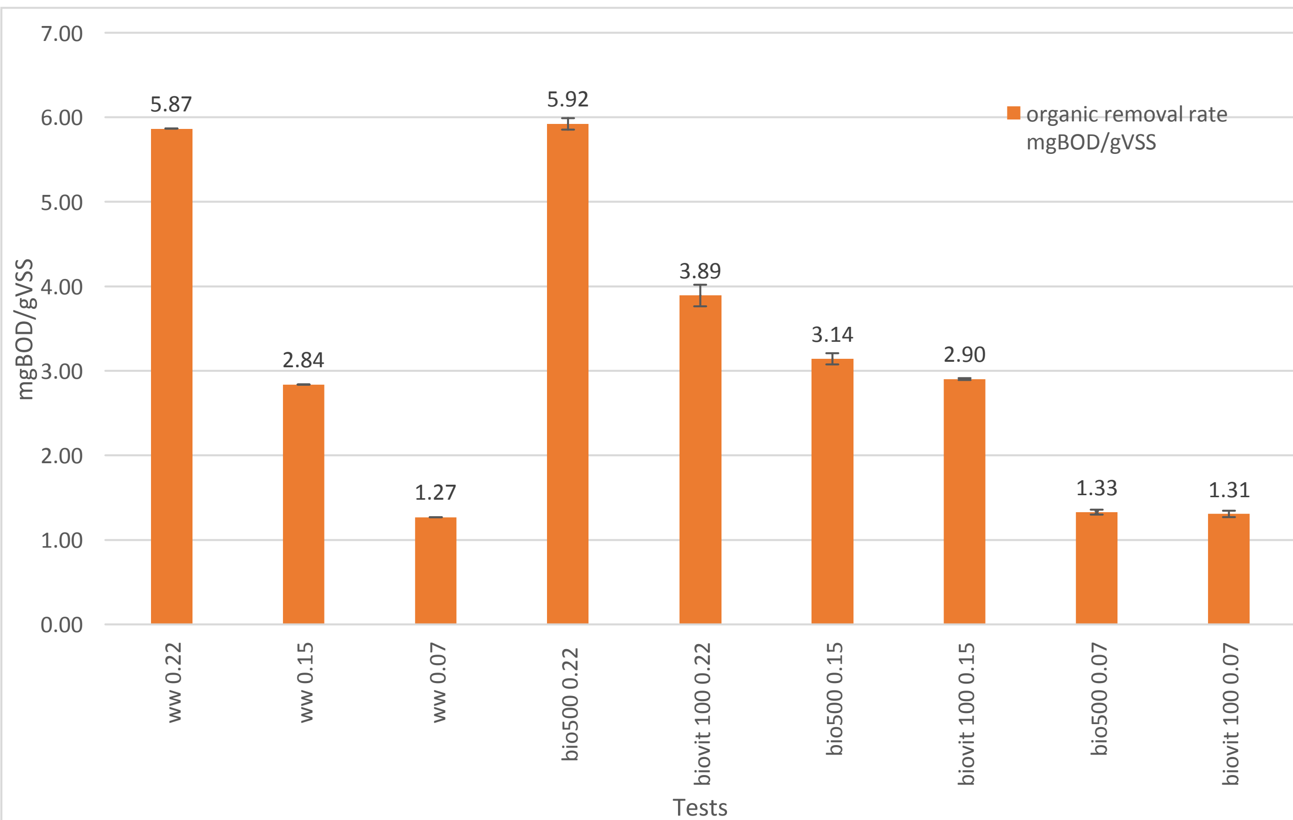


Figure 5- 5: Effect of Biostreme and vitamin addition in different organic loading rates on organic removal rates

A similar behavior was observed with the addition of biostreme, 500 ppm of the trace metals solution resulted in slight increase in organic removal rates comparing to the control tests, however combination of the biostreme and vitamins solution had a negative effect on the removal rate.

5.4. Modeling results

BOD is the amount of biologically consumed dissolved oxygen throughout oxidation process in wastewater treatment. Readily biodegradable organic carbon and ammonia matters are commonly the main source of BOD as cBOD and nBOD respectively.

There are more than a few standard methods for BOD qualification in wastewater, which approximates the kinetics of dissolved oxygen consumption by microorganisms present in the waste stream. These methods are built on mathematical model simulating the wastewater quality.

BOD which is also referred to as biological oxygen demand is based on microbial oxygen consumption through the aerobic oxidation of the electron donor as the carbon and ammonia sources in wastewater;

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Equation 10: Organic removal with glucose as carbon source

$$NH_3 + 2O_2 \rightarrow NO_3^- + H_2O + H^+$$

Equation 11: Ammonia oxidation equation

cBOD is the proportion of the oxygen consumed in the carbonaceous compounds oxidation to mainly CO_2. Formulation of cBOD alteration is done by mainly simplified kinetics as following;

$$d[DO]/dt = d[cBOD]/dt = -k[cBOD]$$

Equation 12: Dissolve oxygen removal

where k is the first order reaction rate constant usually d^{-1}. The equation after integration is;

$[cBOD] = [cBOD]_0 * e^{-kt}$

Equation 13: First order BOD removal

In which the $cBOD_0$ is the initial carbonaceous BOD concentration. The ultimate BOD concentration as well as the BOD removal rate is estimated through a $[BOD_5]$ first order exponential value where;

$[uBOD] – [cBOD] = [BOD_5] * (1-e^{-kt})^{-1}$

Equation 14: First order BOD removal

After modeling the batch test BOD results from the BOD trak II device the generated results were extremely close to the experimental outcomes. As an illustration, some of the modeling results and experimental results are shown in the following graphs;

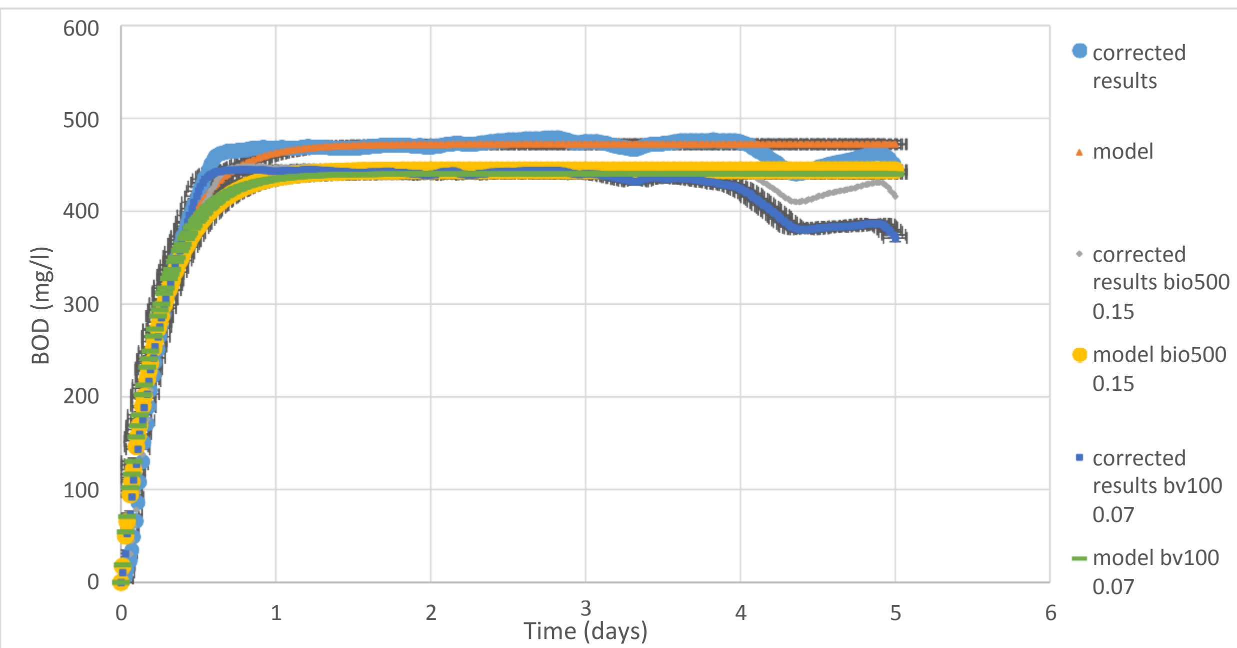


Figure 5- 6: Comparison between modeled data and experimental data

Based on the comparison graphs, the results from different configuration with diverse additive concentrations and organic loadings the experimental data seamlessly matched the simulated BOD results. The data from three different organic loading and diverse concentrations of additives were simulated.

5.5. Conclusion

Both additives and organic loading rates are important factors in BOD activated sludge batch tests. Addition of 500 ppm of the biostreme as an assorted trace metal mixture slightly improved the BOD removal rate while combination of same amount of the biostreme with 100 ppm of the vitamins mixture did not boost the process.

Alteration in organic loading had a noticeable effect on the BOD removal rate, where addition of more seed or in other words decreasing the F/M ratio, significantly deteriorated the BOD removal rates.

Chapter 6: Influence of biostreme additives on the Colloids and particulates matters under high organic loadings rates

6.1. Introduction

Based on the apprehensions concerning public health and environment, there are number of conventional wastewater treatments developed for chemical and microbial pollutant's elimination.

There are new challenges growing every day which affect the efficiency of these processes. Some of these challenges are; the public wish for high quality water due to increasing knowledge and more informed individuals, the lessening water resources, industrial expansions and rapid population growth worldwide. All these factors lead to development and implementation of new advanced treatment processes (Implications Author et al., 1985).

The important contaminants in wastewater have a size range of between 0.001 to 100 micrometers. Organic materials with sizes less than 0.1 micrometer are mostly cell fragments, viruses, macro elements and diverse debris. Macromolecules cover different groups of solids in wastewater; proteins, lipids, polysaccharides and nucleic acid, moreover solids with smaller sizes include amino acids, fatty acids, carbohydrates, etc.

Contaminants in wastewater are further separated into four main size fractions based on sedimentation, centrifugation and filtration; settleable, supra-colloids, colloids and soluble matters (Implications Author et al., 1985).

Table 6-1: Diverse solids in wastewater stream

Solid fractions	Size range (micrometer)	COD % fraction	TOC % fraction
Soluble	<0.08	25	31
Colloids	0.08-1.0	15	14
Supra-colloids	1-100	26	24
Settleable	>100	34	31

One of the critical issues in wastewater treatment processes including advanced treatments such as membrane bioreactors, is presence of different categories of solids in the waste stream which require several stages to be removed.

All wastewaters consist of two main parts; the solid and the liquid components. The first phase of the removal process focuses on extensive solid removal which covers different stages such as; filtrations, sedimentation, clarification, etc (Barjoveanu & Teodosiu, 2010) (Implications Author et al., 1985).

Settleable solids are bulky constituent parts removed by screening and settling processes. Colloids are dispersed in the waste stream which is not a chemical bound but it is a result of surface charges that form stability for the particles in the wastewater solution.

As solids present in wastewater get as agile and small as colloids, substantial separatory issues derive where even advanced treatments such as membrane bioreactors face problems such as fouling because of colloids existence in the influent stream (Jarusutthirak & Amy, n.d.).

The efficiency of conventional activated sludge wastewater treatment process is restricted by the suspended solids which require large settling tanks. Advantages of membrane bioreactors are higher biomass concentrations, higher BOD and COD removal rates, less sludge production, etc. Although there are many benefits to using membrane bioreactors, but high cost and failure due to fouling are two of the negative aspects of this advanced process (Spencer & Sc, n.d.).

Hydrophilic organic colloids, have a remarkable share in the fouling and flux decline in advanced wastewater membrane processes.

Presence of this category of solids in wastewater effluent not only influences the final COD, BOD and TOC, but it forms limitations for reuse applications. A chief loss of efficiency due to membrane fouling is an extensive matter concerning colloids existence in the wastewater's effluent.

As microorganisms are the centre of organic matter composition, operational factors affecting them have received exclusive consideration in the recent years. A diversity of factors has the possibility to change the growth curve and performance production of the microorganisms (Implications Author et al., 1985).

To enhance the biological wastewater treatment a diversity of characteristics must be measured. Parameters that affect the efficiency can vary from temperature and pressure to the nutrients additions. Low degradation and solid removal rates might be an outcome of micronutrients restriction moreover, one of the dominant resolutions for this low pace, is addition of microelements.

Except macronutrients such as C, H, N, O, S and P, obtainability of satisfactory quantities of micronutrients can have a significant effect on the metabolic rate associated with the microorganisms. These essentials support the growth of microorganisms and can be separated into two main types; Metals (ions) and vitamins (Burgess, Harkness, et al., 2000).

Commonly, a complex assortment of nutrients is required in biological treatment processes. This combination regularly comprises of mutually the macronutrients and micronutrients. Absenteeism of some explicit nutrients can result in unstable Bio systems along with decrease in microbial growth and treatment effectiveness.

In this chapter the effect of micronutrients, trace metals and vitamins, supplementation on biological organic removal process efficiency was studied through specific batch tests. COD removal rates along with the solids removal with a special focus on colloids and particulates were the central concern of this part of the research project. The focus of this part of the study was on RAS as the seed while using different loading rates.

Since a dissimilar behaviour was observed when the system was fed with RAS as the seed, a different setup was employed to study both the effects of the micronutrients and different F/M ratios on the colloids and particulates concentrations after five days of BOD removal.

6.2. Materials and methods

With the aim of assessing the effect of biostreme and vitamin's solution as well as the F/M ratio on biodegradability, BOD batch tests with three different F/M ratios and single concentration of biostreme individually and in a combination with the vitamin's solution were conducted.

The additives applied were specific complex mixture of trace metals stated as biostreme and a complex mixture of mostly group B vitamins concentrations. 500 ppm of the biostreme and in a combination with 100 ppm of the vitamins were supplemented to experimental setup.

The composition of the biostreme (trace metals) and the vitamin's solution is as shown in the following table:

Table 6-2: Composition of biostreme and vitamin solution

Additive	**formula**
Biostreme	$CoSO_4.H_2O$
	$CuSO_4.5H_2O$
	$FeSO_4.7H_2O$
	KCl
	$MnSO_4.H_2O$
	MoO_3
	$NiCl_2.6H_2O$
	$ZnSO_4.1H_2O$
	H_2SeO_3
	$Na_2WO_4.2H_2O$
	$VO.SO_4.5H_2O$
	$Na_2B_8O_{13}.4H_2O$
Vitamins	B_3
	B_5
	B_7
	Group of B
	Group of A
	C

The biostreme is mainly a mixture of metals salts; iron, copper, sodium, magnesium, manganese, molybdenum, cobalt, nickel with approximately 80% water.

BOD trak II was used in which the BOD is measured through a physical method deprived of any chemical measurements. The Hach BOD Trak II is simple to arrange and allows for quick sample preparation for BOD analysis. Basically, a known volume of wastewater sample and nutrient buffers were transferred into each of six BOD Trak II bottles and the bottles were connected to the instrument's pressure sensors in addition a measurement range was selected from the device list and incubated.

Factors such as temperature, wastewater composition and source, aerated water nutritional buffers, aeration method and dilution factor were persistent in entire tests, except the extra trace metals and vitamins concentrations and the F/M ratio which was associated with the seed concentrations.

Each bottle consisted of three main components; seed, sample and aerated water. As detailed in previous chapter the sample of all the batch tests were municipal wastewater collected from Humber treatment plant, Toronto, Ontario. The seed used for this biodegradability test was RAS from the same source as the sample, while aerated water was 48-hour aerated distilled water augmented with precise concentrations of nutritional buffers.

Continuous stirring in the flasks provided extra oxygen to the sample and made greater food resources available to the bacteria. This resulted in faster respiration and ingestion of oxygen.

BOD Trak II has a graphic display that continuously posts the results. The instrument plots a curve of BOD over time. The device automatically stops the test and stores the gathered data subsequently after the chosen test length of five, seven, or ten days.

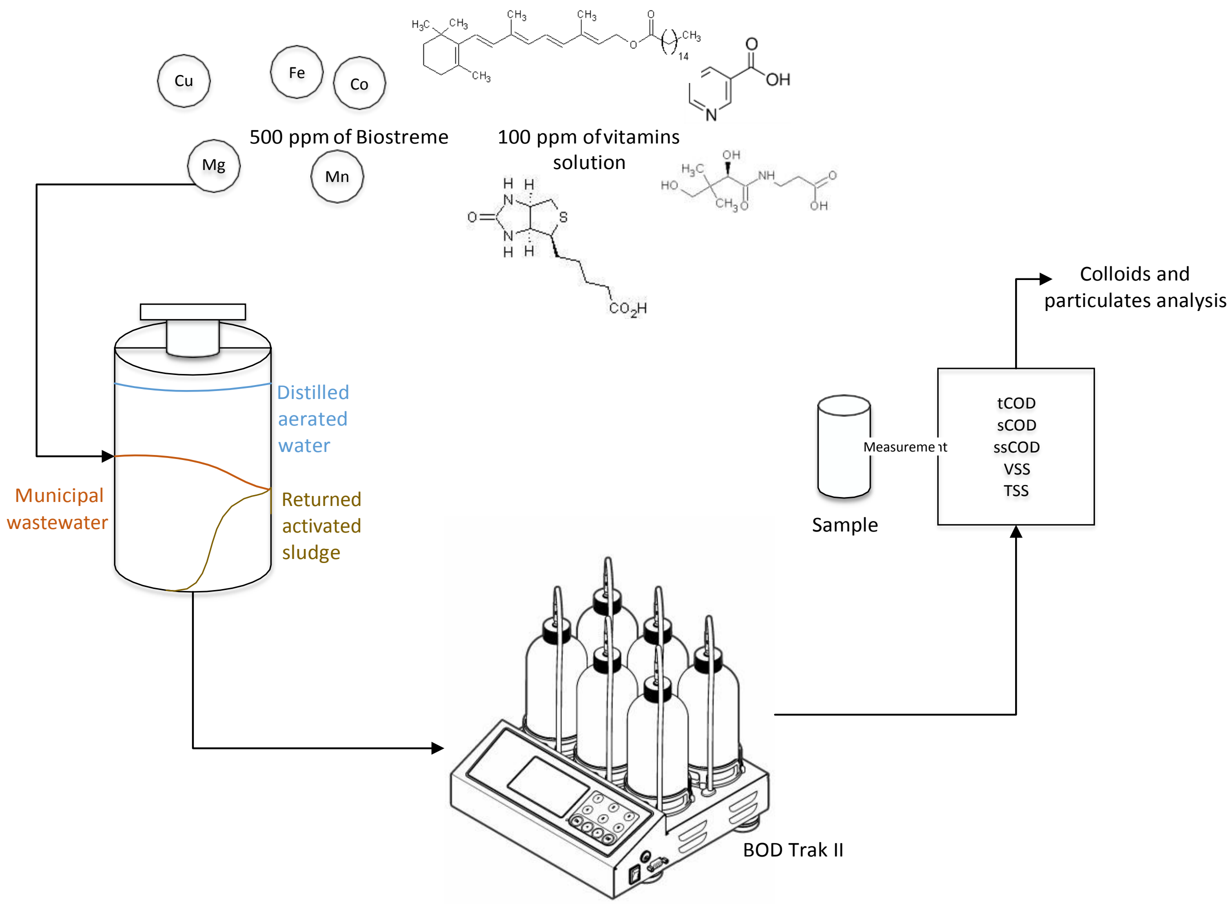


Figure 6- 1: System setup, BOD device configuration, test bottles components

The BOD Trak II device measures BOD using the respirometric pathway. all test bottles are connected to a pressure sensor in a closed arrangement. As bacteria consume oxygen in the sample, the pressure in the bottle headspace drops. This pressure change associates straight to BOD. Through measuring pressure fluctuations as an alternative to dissolved oxygen concentrations, the necessity for probes and titrations is abolished. (figure 6-1)

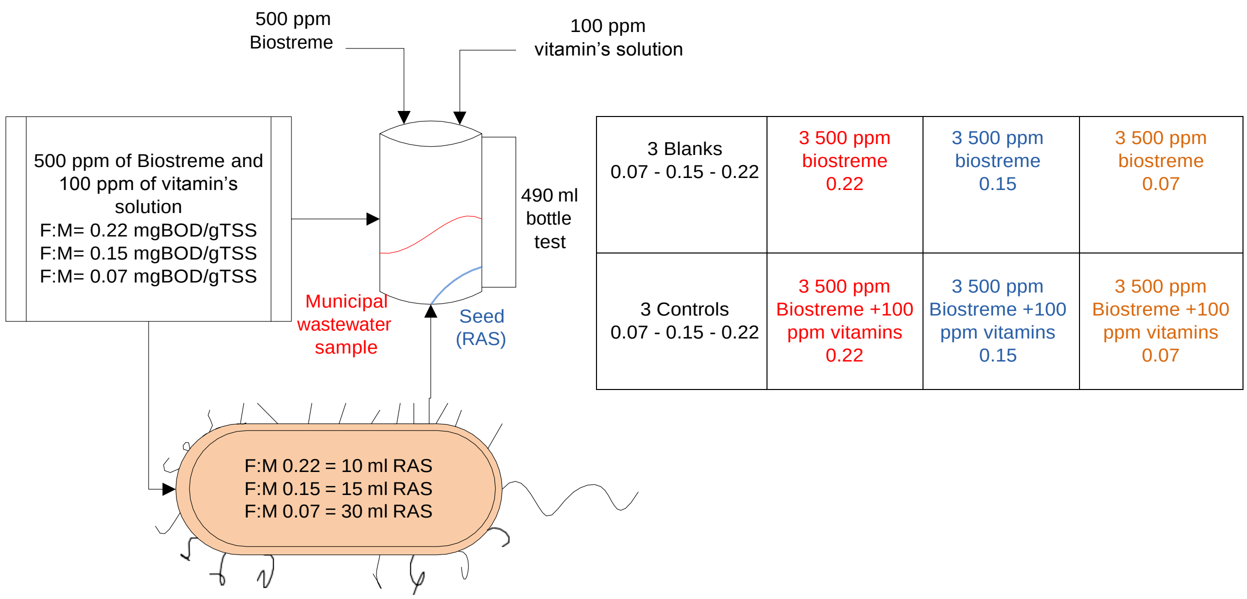


Figure 6- 2: Experimental system setup with different organic loadings and additives concentrations

The F/M ratios applied to the experiments were; 0.22, 0.15 and 0.07 mgBOD/gTSS which involved 10, 15 and 30 ml of seed correspondingly. All analysis contained 110 ml of municipal wastewater and a specific volume of aerated distilled water to reach the specific dilution factor of 1.45. (figure 6-2)

The setup consisted of 24 separate test bottles, three main components were added and the pressure sensors were placed on top of the bottle's lid. After five days of operation all tests were stopped and individual samples were collected from each test bottle.

The municipal wastewater's (sample), total and soluble COD, VSS were measured before the test's primary startup. Both COD and VSS measurements were arranged on unfiltered samples with standard methods for wastewater analysis.

Each sample collected from the test bottles was analyzed in the COD content. The Total COD (tCOD), Soluble COD (sCOD) and Semi Soluble COD (ssCOD) were measured in each sample moreover, the volatile suspended solids (VSS) and the total suspended solids (TSS) were measured with the aim of data normalization.

6.3. Results and discussion

The effect of three different organic loading rates was evaluated using five-day BOD tests individually and with addition of 500 ppm of the biostreme solution and 100 ppm of vitamin's mixture. The results from the COD measurements indicate an alteration in removal rates based on the changing factors.

Table 6-3: COD removal rates with different loading rates and additives

tests	tCOD REM AVG %	sCOD REM AVG %	ssCOD REM AVG %
WW 0.22	71	90	87
WW 0.15	63	90	89
WW 0.07	42	90	89
BIO 500 0.22	72	92	92
BIOvit 100 0.22	68	93	93
BIO 500 0.15	68	93	92
BIOvit 100 0.15	65	92	92
BIO 500 0.07	46	89	89
BIOvit 100 0.07	44	89	89

The results stated in table 6-3 show a decrease in the total COD removal rates when the system was fed with higher volumes of the seed or in other words when the test was conducted with lower organic loading rates. The total COD removal rate had a 40% decline when the F/M ratio was decreased from 0.22 to 0.07 mgBOD/gTSS.

Addition of 500 ppm of the biostreme slightly increased the removal rates by less than 5%, while combination of the biostreme and 100 ppm of the vitamins solution resulted in a higher tCOD removal in F/M ratios of 0.15 and 0.07 mgBOD/gTSS.

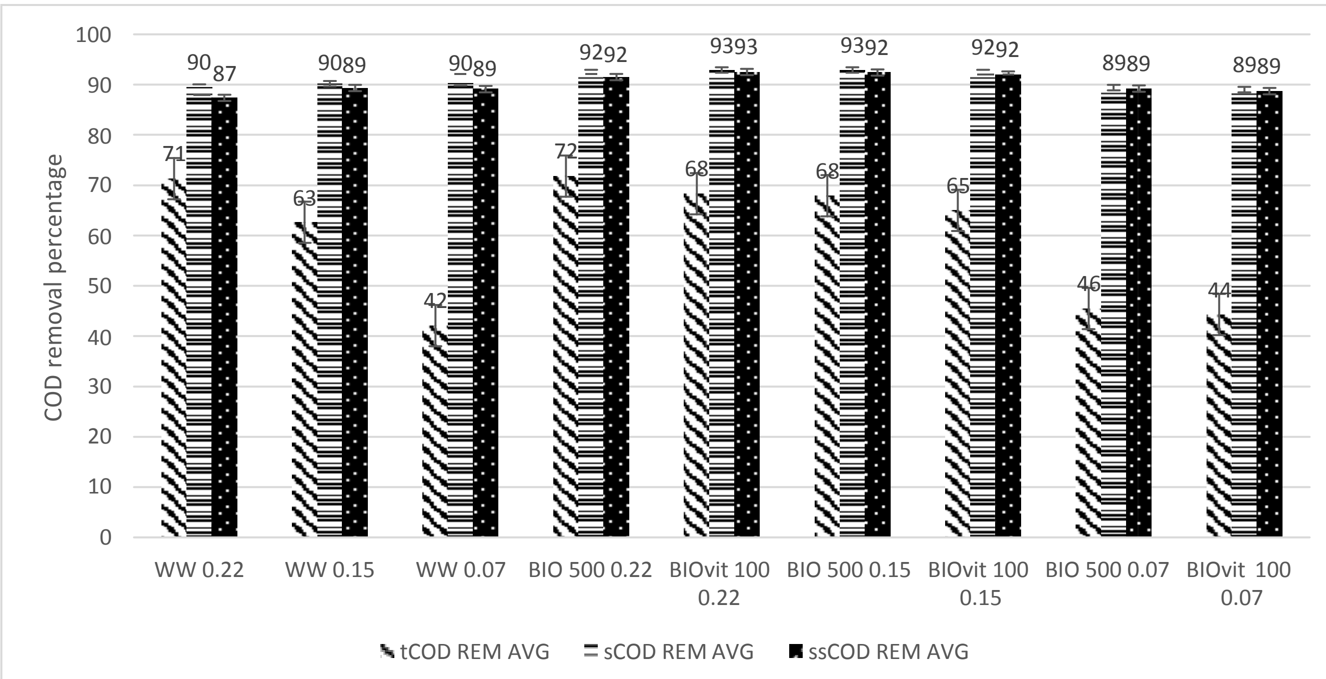


Figure 6- 3: COD removal comparisons between different F:M ratios and additives

Comparison between the soluble and semi soluble COD's indicated that, addition of more RAS to the test bottles or sample supplementation with trace metals and vitamins had no considerable impacts on the removal rates.

Table 6-4: Colloids and particulate concentrations in different loading rates and additives

tests	**COLLIODALS AVG**	**PARTICLES AVG**
WW 0.22	17.0	144
WW 0.15	10.0	325
WW 0.07	19.0	730
BIO 500 0.22	6.7	162
BIOvit 100 0.22	3.0	195
BIO 500 0.15	5.0	294
BIOvit 100 0.15	4.3	323
BIO 500 0.07	2.7	664
BIOvit 100 0.07	4.0	676

The colloids and particulates concentrations in the samples indicate a decrease in the colloids concentrations when the system was supplemented with 500 ppm of the biostreme.

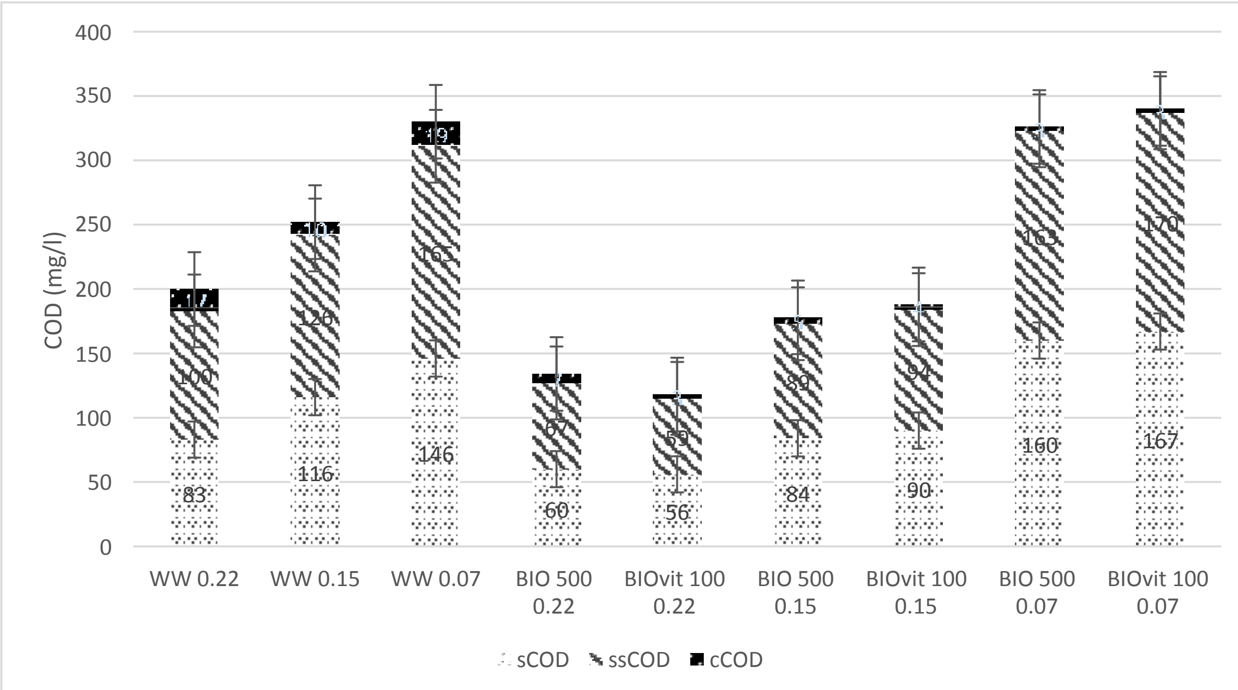


Figure 6- 4: Biodegradability analysis in test with different organic loadings and additives

The results from the 0.22 mgBOD/gVSS loading rates show a 10 mg/l drop in colloids concentration when the control system was supplemented with 500 ppm of the biostreme, in addition mixture of the biostreme and 100 ppm of the vitamins solution resulted in 82% decrease in the colloids concentration. (figure 6-4)

The same scheme was observed in all F/M ratios where addition of micronutrients had a significant influence on the colloids removal rates.

Regarding the particulates matters addition of more RAS resulted in higher concentrations, where by adding 20 ml of RAS to the 0.22 mgBOD/gVSS test the particulates concentrations augmented from 144 to 730 mg/l.

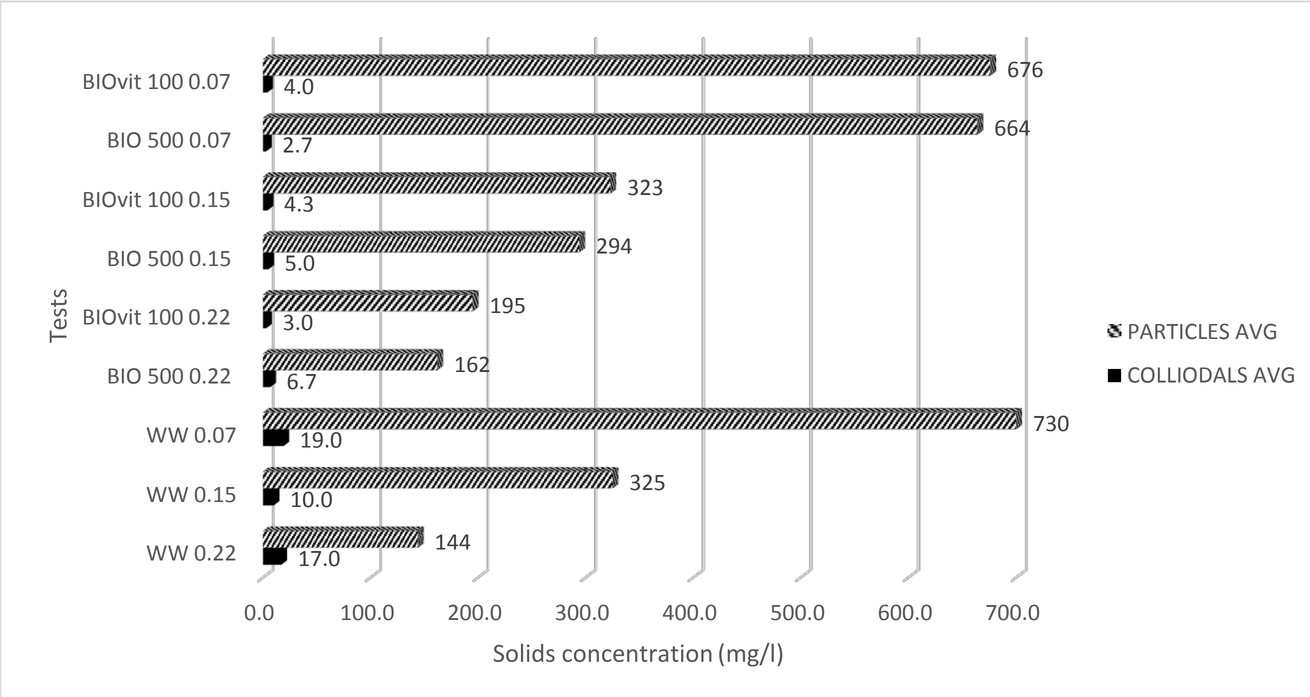


Figure 6- 5: Particulate and colloids concentrations in different F:M ratios and additives concentrations

There are no specific trends observed concerning the particulates, however addition of biostreme and vitamins reduced the concentrations in F/M ratios of 0.15 and 0.07 mgBOD/gVSS.

6.4. Conclusion

The results indicate that;

- Decrease in organic loading rate leads to less tCOD removal rates.
- There were no specific trends recognized in the sCOD and ssCOD removal rates.
- The colloids analysis shows a specific trend; increase in seed volume resulted in higher colloids concentration in the control tests, on the other hand,
- Biostreme and vitamin supplementation decreased the colloids concentration extensively.

Chapter 7: Conclusion and Future works

Biological mechanisms are the core part of biological wastewater treatment processes. In these specific processes the organic matter is converted to mineralized products by means of living microorganisms present in the waste stream.

The main microorganisms involved are bacteria, protozoa, fungi, algae and worms, which have different characteristics and features. The most dominant organism responsible for biological treatment is bacteria.

Bacteria, as a specific group of microorganisms are the main reason for the simultaneous chemical processes occurring in the system referred to as metabolism. In other words, growth and reproduction of organisms, energy storage and cell synthesis are the main parts of bacterial metabolism; catabolism and anabolism.

The main objectives of recent biological treatment advancements are operational control and higher rates. Achieving both goals can be based on several different external and internal factors; one of many is the nutritional balance and supplementation for involved microorganisms.

Major elements such as carbon, hydrogen, nitrogen, phosphorus and sulphur are required in large portions while metals such as cupper, magnesium, iron, sodium, potassium, etc. as well as diverse vitamins comprising of vitamins group B are a few of the minor elements required by microbial population in the treatment process. Most of the mentioned microelements have key roles as enzyme activators and cofactors.

Based on this study, biological organic removal process which is commonly referred to as activated sludge process, requires an assorted complex of micronutrients; both trace metals and vitamins for better organic and solids removal rates.

Biostreme, a specific solution of diverse trace metals concentrations, as well as a vitamin solution was evaluated through different organic removal batch tests done with BOD Trak II device. The main objective of the tests was to evaluate the effect of micronutrient supplementation on both the organic removal and colloids and particulates concentrations. The results indicate that, different configurations demonstrate that in average, addition of 500 ppm of the biostreme had the maximum rate of organic removal. It can be concluded from the BOR tests that RAS has a to some extent dissimilar behavior comparing to the AS and the yeast. Supplementation with 200 ppm of the biostreme had the lowest yield amongst all tests. Results from the solids analysis indicate that trace metals and vitamins supplementation can beneficially affect the colloids and particulates removal process. Tests done with AS and yeast as their seed show a constructive reaction to addition of 100 and 500 ppm of the biostreme as well as mixture of the biostreme and vitamin's solution, while the system was slightly different with RAS.

Alteration in additives and organic loading rates can result in noticeable changes in organic and solids removal rates. Addition of 500 ppm of the biostreme, slightly enhanced the BOD removal rate while combination of the biostreme with 100 ppm of the biostreme did not boost the process. organic loading affected the BOD removal rate, where decreasing the F/M ratio, considerably declined the BOD removal rates. Decrease in organic loading rate leads to less tCOD removal rates while, biostreme and vitamin supplementation decreased the colloids concentration extensively.

The results from the additives used in this study which were a mixture of specific concentrations of trace metals and vitamins show beneficial effects on colloids and organic matter removal rates in specific cases. For better achievements in future, it is suggested to create an optimized solution with a mixture of beneficial trace metals to the BOR process. The results should also be studied through continuous processes besides the batch tests. In continuous processes this additive might affect the influent of the process extensively since considerable effects were observed in 5 days' batch tests. It is also important to expand this study to other biological processes used in treatment plants other than activated sludge system.

References

A. C. Anthonisen, R. C. Loehr, T. B. S. Prakasam, and E. G. S. (1976). Inhibition of Nitrification by Ammonia and Nitrous Acid on JSTOR. Retrieved November 12, 2015, from http://www.jstor.org/stable/25038971?seq=1#page_scan_tab_contents

Aleem, M., & Alexander, M. (1960). Nutrition and Physiology of Nitrobacter agilis'. Retrieved October 1, 2015, from http://aem.asm.org/content/8/2/80.full.pdf

Barjoveanu, G., & Teodosiu, C. (2010). Modelling and simulation of UF process for the removal of suspended solids and colloids from wastewaters (PDF Download Available). Retrieved from https://www.researchgate.net/publication/269219602_Modelling_and_simulation_of_UF_process_for_the_removal_of_suspended_solids_and_colloids_from_wastewaters

Burgess, J. ., Quarmby, J., & Stephenson, T. (1999a). Role of micronutrients in activated sludge-based biotreatment of industrial effluents. *Biotechnology Advances*, *17*(1), 49–70. http://doi.org/10.1016/S0734-9750(98)00016-0

Burgess, J. E., Harkness, J., Longhurst, P. J., & Stephenson, T. (2000). Nutrient balancing for enhanced activated sludge reactor performance: UK perspective. *Water Science and Technology*, *41*(12). Retrieved from http://wst.iwaponline.com/content/41/12/223

Burgess, J. E., Quarmby, J., & Stephenson, T. (1999b). Micronutrient supplements for optimisation of the treatment of industrial wastewater using activated sludge. *Water Research*, *33*(18), 3707–3714. http://doi.org/10.1016/S0043-1354(99)00094-9

Burgess, J. E., Quarmby, J., & Stephenson, T. (1999c). Micronutrient supplements to enhance biological wastewater treatment of phosphorus-limited industrial effluent. *Process Safety* *and Environmental Protection*, *77*(B4), 199–204. http://doi.org/10.1205/095758299530071

Burgess, J. E., Quarmby, J., & Stephenson, T. (1999d). Role of micronutrients in activated sludge-based biotreatment of industrial effluents. *Biotechnology Advances*, *17*(1), 49–70. http://doi.org/10.1016/S0734-9750(98)00016-0

Burgess, J. E., Quarmby, J., & Stephenson, T. (2000). Vitamin addition: An option for sustainable activated sludge process effluent quality. *Journal of Industrial Microbiology and Biotechnology*, *24*(4), 267–274. http://doi.org/10.1038/sj.jim.2900817

Clark, T., & Stephenson, T. (1998a). Effects of Chemical Addition on Aerobic Biological Treatment of Municipal Wastewater. *Environmental Technology*, *19*(6), 579–590. http://doi.org/10.1080/09593331908616714

Clark, T., & Stephenson, T. (1998b). Effects of Chemical Addition on Aerobic Biological Treatment of Municipal Wastewater. *Environmental Technology*, *19*(6), 579–590. http://doi.org/10.1080/09593331908616714

Cox, J. (1982). Transient in a column reactor : limitations , behaviour and effect of growth on a solid substrate, *14*(1968).

Cyplik, P., Grajek, W., Marecik, R., & Kroliczak, P. (2007a). Effect of macro/micro nutrients and carbon source over the denitrification rate of Haloferax denitrificans archaeon. *Enzyme and Microbial Technology*, *40*(2), 212–220. http://doi.org/10.1016/j.enzmictec.2006.04.003

Cyplik, P., Grajek, W., Marecik, R., & Kroliczak, P. (2007b). Effect of macro/micro nutrients and carbon source over the denitrification rate of Haloferax denitrificans archaeon. *Enzyme and Microbial Technology*, *40*(2), 212–220. http://doi.org/10.1016/j.enzmictec.2006.04.003

ecos. (n.d.). Wastewater Nitrification: How it works. Retrieved April 5, 2017, from http://www.ecos.ie/wastewater-nitrification-how-it-works/

Foglar, L., Briski, F., Sipos, L., & Vuković, M. (2005). High nitrate removal from synthetic wastewater with the mixed bacterial culture. *Bioresource Technology*, *96*(8), 879–88. http://doi.org/10.1016/j.biortech.2004.09.001

Gundersen, K. (1955). Effects of B-vitamins and Ammo-acids on Nitrification. *Physiologia Plantarum*, *8*(1), 136–141. http://doi.org/10.1111/j.1399-3054.1955.tb08966.x

Hem, L. J., Rusten, B., & Ødegaard, H. (1994). Nitrification in a moving bed biofilm reactor. *Water Research*, *28*(6), 1425–1433. http://doi.org/10.1016/0043-1354(94)90310-7

Henze, M., Grady, C. P. L., Gujer, W., Marais, G. V. R., & Matsuo, T. (1987). A general model for single-sludge wastewater treatment systems. *Water Research*, *21*(5), 505–515. http://doi.org/10.1016/0043-1354(87)90058-3

Hu, Z., Chandran, K., Grasso, D., & Smets, B. F. (2002). Effect of nickel and cadmium speciation on nitrification inhibition. *Environmental Science and Technology*, *36*(14), 3074–3078. http://doi.org/10.1021/es015784a

Implications Author, R., Levine, A. D., Tchobanoglous, G., & Asano, T. (1985). Characterization of the Size Distribution of Contaminants in Wastewater: Treatment and Characterization of the size distribution of contaminants in wastewater: treatment and reuse implications. *Source: Journal (Water Pollution Control Federation) Journal*, *5763180147*(7), 805–816. Retrieved from http://www.jstor.org/stable/25042701

Jarusutthirak, C., & Amy, G. (n.d.). Role of Soluble Microbial Products (SMP) in Membrane

Fouling and Flux Decline. http://doi.org/10.1021/es050987a

Jefferson, B., Burgess, J. E., Pichon, A., Harkness, J., & Judd, S. J. (2001a). Nutrient addition to enhance biological treatment of greywater. *Water Research*, *35*(11), 2702–2710. http://doi.org/10.1016/S0043-1354(00)00553-4

Jefferson, B., Burgess, J. E., Pichon, A., Harkness, J., & Judd, S. J. (2001b). Nutrient addition to enhance biological treatment of greywater. *Wat. Res*, *35*(11), 2702–2710. Retrieved from http://ac.els-cdn.com/S0043135400005534/1-s2.0-S0043135400005534-main.pdf?_tid=4c43b5ca-4490-11e7-808c-00000aab0f01&acdnat=1496077400_8e2a8fbd494bb5b39a21722182c3d8a8

Kim, Y. M., Park, D., Lee, D. S., & Park, J. M. (2008). Inhibitory effects of toxic compounds on nitrification process for cokes wastewater treatment. *Journal of Hazardous Materials*, *152*(3), 915–921. http://doi.org/10.1016/j.jhazmat.2007.07.065

Konopka, A., Oliver, L., & Jr., R. F. T. (1998). The Use of Carbon Substrate Utilization Patterns in Environmental and Ecological Microbiology. *Microbial Ecology*, *35*(2), 103–115. http://doi.org/10.1007/s002489900065

Li, Y., Lei, Z., Zhang, Z., & Sugiura, N. (2006). Effects of nutrient addition on phenol biodegradation rate in biofilm reactors for hypersaline wastewater treatment. *Environmental Technology*, *27*(5), 511–20. http://doi.org/10.1080/09593332808618662

Liu, Y.-Q., Wu, W.-W., Tay, J.-H., & Wang, J.-L. (2008). Formation and long-term stability of nitrifying granules in a sequencing batch reactor. *Bioresource Technology*, *99*(9), 3919–22. http://doi.org/10.1016/j.biortech.2007.07.041

Magalhães, C., Costa, J., Teixeira, C., & Bordalo, A. a. (2007). Impact of trace metals on denitrification in estuarine sediments of the Douro River estuary, Portugal. *Marine Chemistry*, *107*(3), 332–341. http://doi.org/10.1016/j.marchem.2007.02.005

Nazaroff, & Alavarez, C. (n.d.). Biological Wastewater Treatment Aerial View of the Water Reclamation Plant in Hanover. Retrieved from http://www.hanovernh.org/Pages/HanoverNH_PublicWorks/WaterRecl/Index

Orhon, D., Ateş, E., Sözen, S., & Çokgör, E. U. (1997). Characterization and COD fractionation of domestic wastewaters. *Environmental Pollution*, *95*(2), 191–204. http://doi.org/10.1016/S0269-7491(96)00111-X

PETER, S. (2008). Biological denitrification of high nitrate industrial streams. Retrieved November 16, 2015, from http://ufdcimages.uflib.ufl.edu/UF/E0/02/22/65/00001/peter_s.pdf

Selimoğlu, F., Öbek, E., Karataş, F., Arslan, E. I., & Tatar, Ş. Y. (2015). Determination of amounts of some vitamin B groups in domestic wastewater treatment plants. *Turkish Journal of Science & Technology*, *10*(2), 1–5. Retrieved from http://fbe.firat.edu.tr/sites/fbe.firat.edu.tr/files/1-5.pdf

Sharma, B., & Ahlert, R. C. (1977). Nitrification and nitrogen removal. *Water Research*, *11*(10), 897–925. http://doi.org/10.1016/0043-1354(77)90078-1

Spencer, P., & Sc, D. B. (n.d.). The Biological Basis of Wastewater Treatment. Retrieved from http://www.s-can.nl/media/1000154/thebiologicalbasisofwastewatertreatment.pdf

Tay, J.-H., Pan, S., He, Y., & Tay, S. T. L. (2004). Effect of Organic Loading Rate on Aerobic

Granulation. I: Reactor Performance. *Journal of Environmental Engineering*, *130*(10), 1094–1101. http://doi.org/10.1061/(ASCE)0733-9372(2004)130:10(1094)

Vanrolleghem, P., Olsson, G., & Spanjers, H. (n.d.). Respirometry in control of the activated sludge process. *Water Science and Technology*. Retrieved from https://www.academia.edu/29993890/Respirometry_in_control_of_the_activated_sludge_process

Vollertsen, J., & Hvitved-Jacobsen, T. (n.d.). Biodegradability of wastewater – a method for COD-fractionation. Retrieved from http://www.szennyviztudas.bme.hu/files/Biodegrability of wastewater.pdf

Wakefield, J. H. (n.d.). Understanding Separation Essentials For Wastewater Treatment. Retrieved June 26, 2017, from https://www.wateronline.com/doc/understanding-separation-essentials-for-wastewater-treatment-0001

Wang, J. (2012). Fundamentals of Biological Processes for Wastewater Treatment. *Biological Sludge Minimization and …*. Retrieved from http://content.schweitzer-online.de/static/content/catalog/newbooks/978/047/076/9780470768822/9780470768822_Excerpt_001.pdf

Wilkie, a., Goto, M., Bordeaux, F. M., & Smith, P. H. (1986). Enhancement of anaerobic methanogenesis from napiergrass by addition of micronutrients. *Biomass*, *11*(2), 135–146. http://doi.org/10.1016/0144-4565(86)90043-0