LOW COST AUTOMATED ON SITE SYSTEM FOR GROWING AND DISPENSING VEGETATIVE BACTERIA

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ABSTRACT

Microorganisms have been utilized for many years to enhance the bioremediation process of municipal, industrial and agricultural wastewaters. Vegetative bacteria are key to the breakdown of organic matter collected from these waste streams. Historically, bacteria have been available commercially as dormant/resting spores and at relatively low activity levels. As a consequence of these limitations, it has been economically prohibitive in many systems to utilize appropriate levels of bacteria required to significantly reduce Biochemical Oxygen Demand (BOD) and Fats, Oil and Grease (FOG). This system affords the ability to deliver *Pseudomonas* and *Bacillus* species on site at high concentrations.

Extensive research and engineering development conducted by NCH Corporation has resulted in a patented, onsite fermentation technology. This system generates and delivers 3×10^{13} vegetative bacterial cells every 24 hours, allowing effective and economical treatment of wastewater systems that previously were not candidates for biological treatment of BOD and FOG.

The BioAmp® delivers the equivalent of 25-50 lbs. of commercially available dry bacterial bran powder every 24 hours. This is accomplished at a cost of less than 10% of comparable quantities of dry powder. With this new technology, efficacy data has been generated in numerous case studies that demonstrates reproducible BOD reduction compared to baseline data. This type of data generation in a standard format is new to the industry.

The BioAmp® growth vessel is charged daily with water and tablets that contain nutrients and bacterial inoculants. These bacterial strains have been selected for their ability to catabolize starches, proteins, sugars, hydrocarbons, animal fats, cooking greases and oils. Air is supplied continuously to the growth vessel to support aerobic bacterial generation, while dissolved oxygen is maintained within the vessel. The bacteria grow and reproduce as they are continuously recirculated via a pump which injects the liquid culture at a tangentially directed flow into the growth vessel. It is this tangential flow that creates a vortex and suppresses foaming. At the end of the 24 hour growth cycle, the highly concentrated bacteria are automatically discharged to a desired location. Once the growth vessel is emptied, the process is repeated.

KEYWORDS Vegetative, *Pseudomonas*, On-Site, BOD, FOG, Vortex

INTRODUCTION

The automated system described herein is a unique blend of microbiology and engineering. As a result, it is important to describe both the biological and mechanical aspects of this invention.

Bacterial products have been used for many years to enhance bioremediation of wastewater. Two general types of products have been used for over 40 years. One class of product is a stabilized liquid that contains spores (resting bodies) of various *Bacillus species.* The other class of product is a dried, bran based powder traditionally containing several *Bacillus and Pseudomonas species.*

As populations, industrial plants, and commercial operations have increased, the organic loading has also increased in municipal sewer collection systems and wastewater treatment plants. Unfortunately, sewer collection systems, commercial operations, and most industrial plants do not have the luxury of on-site, active biomass to biologically treat their high strength organic waste streams. In many cases, the increase in the number of business operations with high strength waste streams has led to governmental implementation of pretreatment regulations, or at the very least, surcharges for wastewater discharges above set and defined limits for BOD, COD, O&G, and TSS.

The solution to overcoming these issues was to develop an on-site system that would economically, reliably and automatically deliver the proper strains of bacteria capable of degrading a broad range of organic matter present in waste water.

The BioAmp® patented system has achieved these goals and will be expanded upon in the Methodology and Results sections.

METHODOLOGY

BIOLOGICAL

In order for any metabolic or catabolic process to occur the organic substrate must be degraded enzymatically. The first step in the breakdown of an organic molecule depends on an enzyme specific to that molecule type. Carbohydrates, proteins, and fat molecules cannot be directly transferred into a bacteria cell as a substrate/food source. The enzymes that enable the metabolic processes are identified below.

Amylase is any member of a class of enzymes that catalyze the hydrolysis, or splitting, of a carbohydrate molecule into smaller molecules. The smaller molecules are then converted to CO_2 and water via the following pathway:

Carbohydrate	→	Hexoses (e.g. glucose)	→
	Amylase mediated Substrate Hydrolysis		Glycolysis

Pyruvic acid	→ Acetyl – CoA Catabolic Conversions	→ $CO_2 + H_2O$ TCA (Kreb's) Cycle and Oxidative Phosphorylation

(Atkinson, et. al., 1991; Nester, et. al., 1973; Stainer, et. al., 1976)

Proteases, or proteolytic enzymes, break the long chainlike molecules of proteins into shorter pieces (peptides) and eventually into their components, amino acids, via the following pathway:

Proteins	→ Protease mediated Substrate Hydrolysis	Peptides	→ Protease mediated Substrate Hydrolysis
Amino Acids	→ Deamination or Transamination	Pyruvic Acid + Free NH_4^+	→ Catobolic Conversion
Acetyl-CoA	→ TCA (Kreb's) Cycle and Oxidative Phosphorylation	$CO_2 + H_2O$	

Notes:

1. The amino acid, Glutamic acid, forms alpha-Ketogultamic acid via transamination. The alpha-Ketoglutamic acid goes directly into the Tricarboxylic Acid cycle (TCA).
2. The amino acid, Aspartic acid, forms Oxaloacetic acid via transamination. The Oxalacetic acid goes directly into the TCA cycle.
3. The free NH_4^+ predominates in acidic and neutral aqueous environments. As the pH increases, NH_3 predominates and is released to the atmosphere as ammonia gas.

$$NH_4^+ \rightarrow NH_3 + H^+$$

(Atkinson, et. al., 1991; Morrison, et. al., 1969; Nester, et. al., 1973; Stainer, et. al., 1976)

Lipases are fat splitting enzymes that hydrolyze, or split, fats into their component fatty acid and glycerol molecules via the following pathways:

Fat	→ Lipase Mediated Substrate Hydrolysis	1 Glycerol Molecule + 3 Fatty acids	
Glycerol	→ Glycolysis	Pyruvic acid	→ Catabolic Conversions

Acetyl – CoA	→ TCA (Kreb's) Cycle and Oxidative Phosphorylation			$CO_2 + H_2O$
<u>Fatty acid</u>	→ Beta-Oxidation	Acetyl-CoA	→ TCA (Kreb's) Cycle and Oxidative Phosphorylation	$CO_2 + H_2O$

(Atkinson, et. al., 1991; Nester, et. al., 1973; Stainer, et. al., 1976)

Most *Bacillus* and *Pseudomonas species* produce protease and amylase enzymes (Atkinson, et. al., 1991; Huijberts, et. al., 1992; Liao, et. al, 1998; Stanier, et. al., 1976; Temple, et. al., 1994: Williams, et. al., 1984). When the bacteria encounter carbohydrates or proteins in their environment they can excrete an extracellular amylase to break down carbohydrates, and they can excrete an extracellular protease to break down the proteins. These smaller molecules can then enter the metabolic pathways of the bacterium.

In the case of fat molecules, certain bacteria can produce a lipase enzyme to break the fat into glycerol and fatty acids. Most *Pseudomonas species* produce lipase, but only a few *Bacillus species* produce lipase (Ahn, et. al, 1999: Frenken, et. al., 1992; Johnson, et. al., 1992; Sonenshein, et. al., 1993; Tan. et. al., 1992; Wiedmann, et. al, 2000; Williams, et. al., 1984). Once fat has been broken down into glycerol and fatty acids, glycerol is then utilized via the normal carbohydrate degradation pathways. Both *Bacillus* and *Pseudomonas* can utilize glycerol (Atkinson, et. al., 1991; Schweizer, et. al., 1996; Williams, et. al., 1984). In fact, a growth medium first developed in 1954, *Pseudomonas* Isolation Agar, contains glycerol as an ingredient (Difco Manual, 1985). However, fatty acid utilization poses a problem for *Bacillus sp.,* even for *Bacillus thuringiensis* which produces lipase.

Most *Bacillus species* cannot utilize fatty acids because they do not have the necessary enzymes to break down the long-chain fatty acids into managable 2-carbon units, the acetyl - Co A unit, that fits into the TCA cycle (Sonenshein, et. al., 1993). For example, the 16-carbon acid, palmitic acid, undergoes seven cycles to yield eight molecules of acetyl – CoA. Each 2-carbon unit, acetyl – Co A, then goes through the TCA cycle (Stainer, et. al., 1976). Some bacteria, i.e. many *Pseudomonas sp,* are able to utilize fatty acids as a carbon and energy source. (Fernandez-Valverde, et. al., 1993). *Pseudomonas species* are very diverse in the carbon sources that they can use for growth, in fact, some species have over 300 different specialized enzymes (Stainer, et. al., 1976). A bacterium can be in a very rich substrate medium, but it doesn't have the enzymes necessary to break that substrate into smaller units. As a result it will not be able to utilize any of that substrate.

Products that contain only *Bacillus species* have a disadvantage in that some *Bacillus species* can degrade fats to glycerol and fatty acids, but only a few *Bacillis species* can degrade the fatty acids. Thus, a *Bacillus* only product may release a great deal of fatty acids which will acidify the environment and prevent the bacteria from growing.

A concern with both liquid and dry bacterial products has always been the lag time required for the bacteria to actually begin degrading and utilizing the organic matter found in waste streams. The *Bacillus* spores in a liquid product are in a dormant, resistant state. To become metabolically active, the spores must first germinate. Germination is a process by which a spore (resting state) becomes a vegatative (metabolically active) cell. This process may take 4-6 hours (Sonenshein 1993). Before *Bacillus* spores in a dry powder can begin functioning, they must first re-hydrate and then germinate into vegetative cells.

The *Pseudomonas* cells in a dry powder are resting at a very low metabolic rate and do not form spores; they must first re-hydrate before becoming active. They are not ready to go as soon as the powder is added to the drain or grease trap.

Our goal was to develop a compact, low-cost, automated system and method to apply large numbers of actively growing bacteria into a drain or waste stream. The result of that work is the patented BioAmp® (Patent Number 6335191 January 1, 2002) which is an on-site fermentor that produces large numbers of actively growing *Bacillus* and *Pseudomonas species.* The bacterial strains used as inoculum for the BioAmp® were chosen for their ability to degrade carbohydrates, proteins, and fats. Additionally, two of the *Bacillus* strains are facultative anaerobes that can grow in the presence of oxygen or without oxygen.

The BioAmp® growth vessel is automatically filled with water and tablets that contain an inoculum of dry *Bacillus* and *Pseudomonas* cultures and nutrients for growth of the bacteria. Air is supplied continuously to the growth vessel. The bacterial culture is continuously circulated via a pump. At the end of each 24 hour growth cycle, the inoculum count has increased by over 4 logs resulting in 3×10^{13}actively growing bacteria delivered to the drain or waste stream daily.

In the environment, bacteria are constantly working to naturally break down organic materials. This natural process generally causes some organic materials to eventually degrade into carbon dioxide and water. Under normal conditions, competition for resources, limited supplies of nutrients, and natural enemies can combine to inhibit rapid bacterial growth. By isolating selected strains of bacteria and providing a food source they prefer, bacteria can be made to multiply at a very fast rate. A large quantity of bacteria can be generated in this manner within a relatively short time. The bacteria can then be used in a wide variety of applications where the breakdown of organic materials is desirable.

One application where the breakdown of organic materials is particularly useful is in the maintenance of grease traps. Grease traps are required on virtually all commercial facilities that discard liquid or solid grease into a sewer system. Grease traps generally range from a capacity of about five gallons to several thousand gallons. The majority of fast food kitchens are equipped with grease traps of about 1000 gallons. The system of drains used for grease traps is generally separate from the drains that carry away waste products from restrooms, spent drinking water, etc. Grease traps tend to collect not only oils and fats, but also various organic waste materials such as starches and vegetable waste materials. Normally, a significant flow of wastewater is also introduced into the separate grease trap drainage system from kitchen drains
where grease is often found. To prevent the wastewater from flushing grease into the city sewer

system, grease traps are designed with a series of weirs that trap the grease within the ontainment vessel and allow wastewater to pass through the vessel on to a city treatment facility.

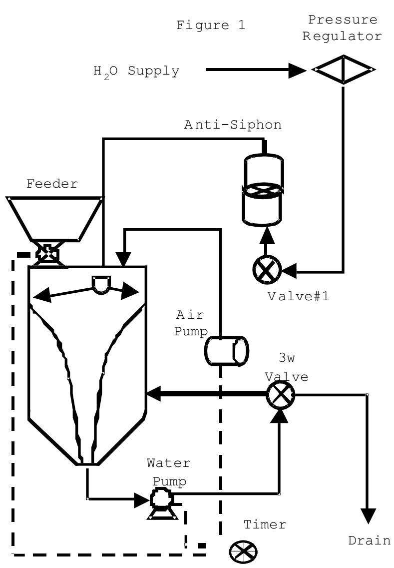
Inevitably, some of the grease in the grease trap passes into the city sewage system downstream from the restaurant. This does not create problems if the amount of grease passing into the sewer system is kept at a low level. Most city standards restrict the release of grease into sewer lines to approximately 250 ppm or less. If signifcant amounts of grease pass into the sewage system, the grease can cause blockages in the city pipes. When this occurs, the grease trap can overflow into the street causing health problems. City maintenance crews often have to dig up the pipes to remove the blockage. The cost of this procedure is typically passed on to the restraurant that released the grease. The restauant usually must also pay a fine. For repeat offenders, the blockage can result in closure of the facility.

MECHANICAL

The present system comprises an automated biogeneration method for producing and dispensing liquid concentrates of active bacteria at predetermined intervals. Bacteria produced in this manner can be used to digest organic material in a variety of waste streams. The system can also be used to supply bacteria for many other useful applications.

A method for growing and selectively discharging bacteria is shown in Figure 1, whereby water and a predetermined quantity of a tableted powdered mixture of dehydrated “starter” bacteria and appropriate nutrient(s) are automatically introduced into a biogeneration chamber for the purpose of growing and quickly multiplying the selected bacteria. Multiple strains of bacteria can be used as long as the nutrient package is designed to support each of the multiple strains. Pressurized air is supplied to the chamber to support aerobic bacterial reproduction, and is introduced via a method using a vortex that controls foaming within the biogeneration chamber. After the mixture is placed in the biogeneration chamber the bacteria is permitted to grow and reproduce for a desired time (about 24 hours). During this process, liquid is continually withdrawn and recirculated from the bottom of the chamber via a pump. This water is reintroduced into the chamber in a tangentially directed flow to create the desired vortex. At the end of the growing period, the active bacteria are preferably discharged from the biogeneration chamber to another holding vessel or, more preferably, directly to a waste stream. Once the contents of the biogeneration chamber are discharged, the process is repeated. The cycle of operation is desirably controlled by an electronic timer having relays that activate and deactivate switches and valves in accordance with predetermined parameters. Significant increases in bacterial production are observed using the system.

FIG. 1 is a simplified schematic view of a biogeneration.



The automated biogeneration system is useful for rapidly growing a large supply of selected strains of bacteria using a small quantity of starter bacteria. The system provides a low maintenance, hands-free process for growing bacteria that, depending upon the bacterial strains selected, are useful in a variety of applications.

The starter bacteria and nutrients needed to support growth and reproduction of the bacteria within the biogeneration chamber are easily transported. This is preferably in a tableted powder form, making the transport of large supplies of onsite bacteria unnecessary. The subject method does not require precise control of the temperature, nor is it necessary to use a filtered air supply or distilled water. Because multiple strains of desirable bacteria can be grown simultaneously in the same biogeneration chamber, they are capable of digesting a variety of organic materials. As a result of being grown onsite, the useful bacteria are active upon introduction into a waste stream.

RESULTS

BioAmp® units have demonstrated excellent efficacy in industrial wastewater and commercial/institutional drain treatments, as well as governing municipal waste treatment facilities. When properly applied to effluent process water, BOD levels can be significantly reduced without the customer allocating major capital expense for physical process improvements.

Some wastewater generators may require wastewater profiling to determine specific application requirements to facilitate desired efficacy and to overcome limiting factors. These applications could include added retention storage (based on flow rate and existing hydraulic retention), wastewater equalization, gross filtration of particulate, pH adjustment, aeration, mixing, supplemental nutrients, dissolved air flotation, identification of major carbon source (pollutant) as well as its concentration, limiting temperature parameters, etc.

Plant effluent levels are monitored prior to installation of the BioAmp® in an effort to establish ‘baseline data’. Historical Plant Effluent Data is also utilized where available for establishing ‘long term baseline data’ which further validates efficacy. Close cooperation with the governing municipal waste treatment facility is maintained throughout the study.

Effluent is tested for the following:

BOD 5-Day	EPA Method 405.1
Total Suspended Solids	EPA Method 160.2
Oil & Grease	EPA Method 1664

Both grab sampling as well as composite sampling methods are utilized throughout the study. Composite sampling comprises critical data accrual and is the main component for reporting, while grab sampling remains important as a real-time guide for adjustment and monitoring.

Special consideration is given to the feed point of bacterial inoculum from the BioAmp® into the waste stream. In most cases, it is beneficial to dispense as close to the 'headwaters' of the effluent flow as possible. This is in an effort to begin the remediation process as far upstream as possible which takes advantage of the in-plant collection system residence time. Establishment of a desirable 'bio-film' in the collection system also aids in the overall early up-stream remediation process. Special consideration of the BioAmp® dispensing sites must be monitored and must maintain supporting temperature ranges, dissolved oxygen levels, pH ranges, absence of 'Clean In Place' or other toxic sanitizing chemical levels to maintain an environment favorable to bacterial growth. Many non-desirable dispense site conditions may be nullified by coordinating dispense time with fluctuating toxic and non-toxic flow conditions.

INDUSTRIAL

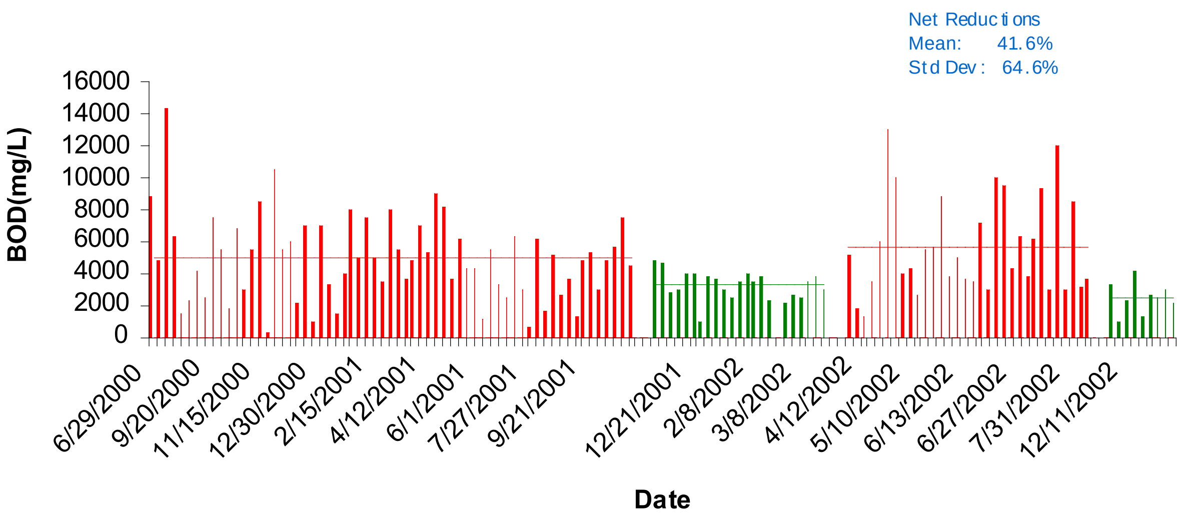
The BioAmp® has been proven to be successful in an application for reduction of high strength BOD effluent levels in a major food manufacturer for mixed foods (salad dressings, barbecue sauce, citrus drinks, etc.). The following efficacy data from the above referenced mixed foods process waste stream was performed with no change to plant process or waste treatment system. Manufacturing and process was active 24/7.

Yearly average flow rate is 180,000 gallons per day. Eight (8) Biogenerator units are in-place with 2 units installed in a remote upstream plant lift station for salad dressing effluent which then feeds to the pH adjustment. The reason for treating the upstream lift station is to take advantage of its retention time prior to the 16,000 gallon skimming tank.

The remaining 6 units dispense individually at 4 hour intervals immediately downstream of pH adjustment and upstream of the 16,000 gallon skimming tank. Retention time for process water which enters the skimming tank can vary from 1 to 3 hours depending on flow rate at a given time.

Therefore, all reductions at this facility are achieved with retention times of 1 to 3 hours without further process improvement.

Figure 2 – BOD vs Time



Actual Mixed Foods data for current BioAmp® installation is shown in figure 2. As evidenced on this graph, the first group of red bars signifies historical plant effluent data collected from 6/29/00 to 10/19/01demonstrating a mean of 4856 mg/L. Following initiation of the BioAmp® program, as evidenced by the following green bars from 11/20/2001 to 3/28/2002, there is a reduction of the mean to 3209 mg/L.

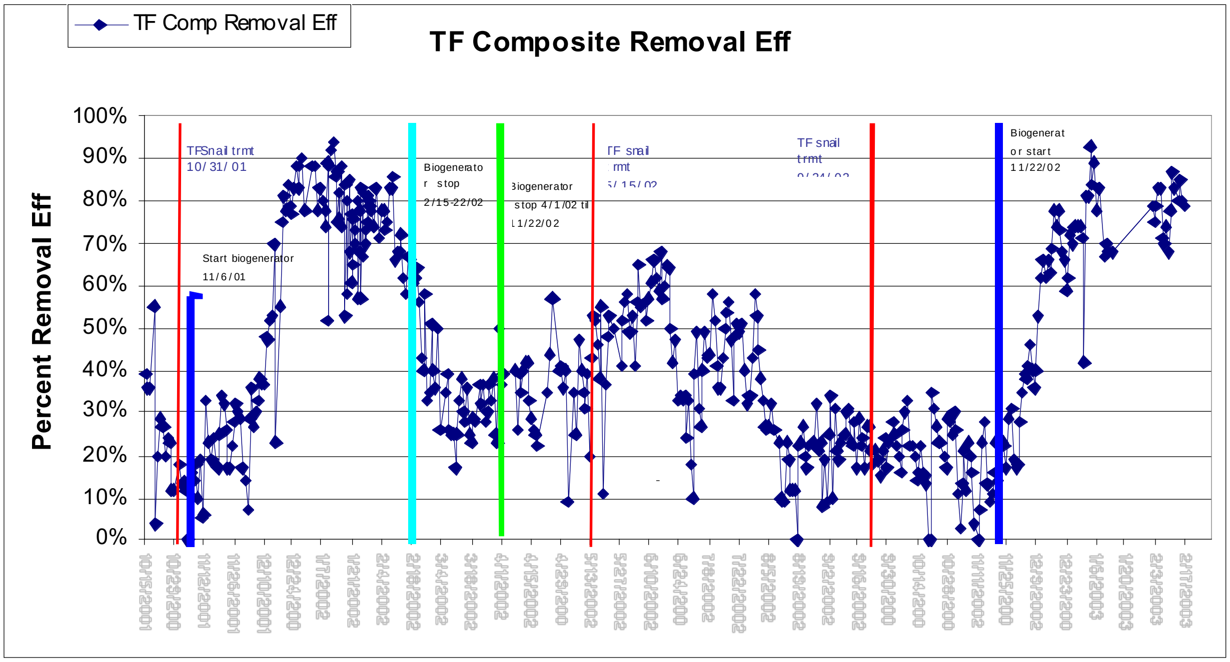
In an effort to verify the above decrease in mean BOD, this study was repeated by inactivating the BioAmp® and collecting data to establish the baseline once more. This is shown in the 2nd red section of the bar graph demonstrating a mean of 5606 mg/L. As evidenced in the green bars of the graph following reactivation of the BioAmps®, BOD mean dropped to 2436 mg/L.

The overall net results in Figure 2 reflect a 41.6% reduction in BOD effluent levels. Additionally, system variability was as evidenced by a 64.6% reduction in standard deviation from the mean. The application of the BioAmp® System in this facility resulted in net savings of $90,000/yr.

<u>MUNICIPALITY</u>

As referenced above in the Industrial Wastewater application, close cooperation and communication was maintained throughout the application with the receiving wastewater treatment facility. This was originally in an effort to validate efficacy and ultimately to reduce water usage surcharge levels via reduction in BOD effluent levels at the mixed food industrial facility. The following study was performed in conjunction with the receiving wastewater treatment facility. The ammonia removal levels experienced at this plant was attributed to the BioAmps® placed up stream at the mixed food plant.

Figure 3 – TF Composite Removal EFF (Benefit to Receiving Waste Treatment Facility)



As shown in Figure 3, this plant utilizes a trickling filter system designed for efficiencies of 80% to 90% ammonia removal. During the past five years, average ammonia removal efficiencies have been 20% to 30%. In the first month following start-up of the BioAmp®, an 80% ammonia removal was achieved.

Efficient removal of ammonia ceased on or about 2/18/2002 as evidenced in Figure 3. This coincided with the inactivation of the BioAmps®. Ammonia removal remained low for the following six months during which BioAmps® remained inactive.

The BioAmps® were reactivated on 11/22/02 and an immediate improvement in ammonia removal to levels of 80% were again attained and maintained as evidenced in Figure 3.

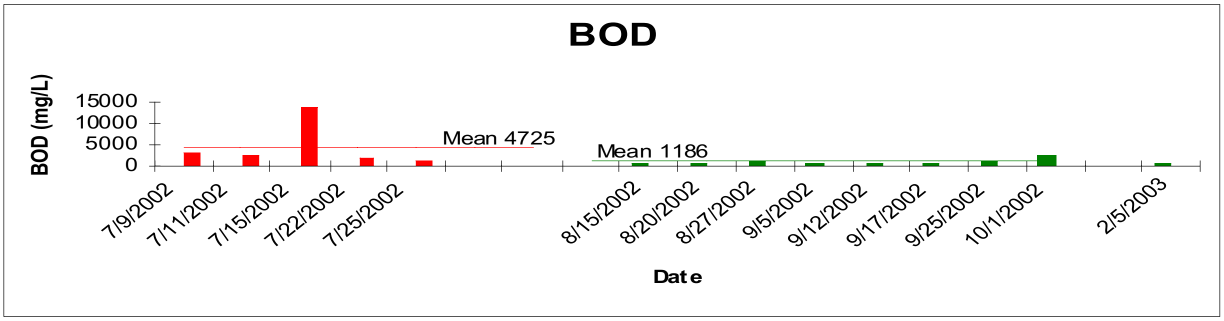
<u>COMMERCIAL</u>

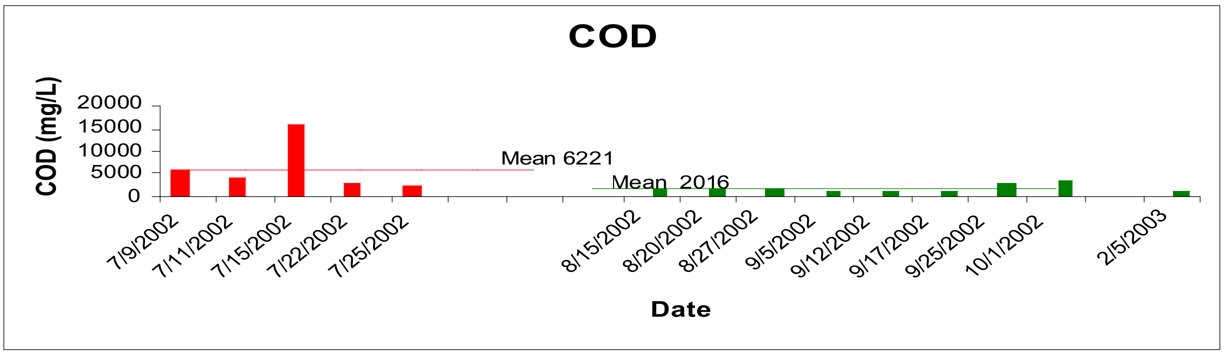
A comprehensive study was performed for a major retail grocer. Four facilities were involved in the study with the data from one store shown in Figure 4. The red bars for each measurement represent the tested effluent from the grease trap prior to pump-out as well as before treatment with the BioAmp. Baseline means are significantly elevated for BOD, COD, TSS and O&G.

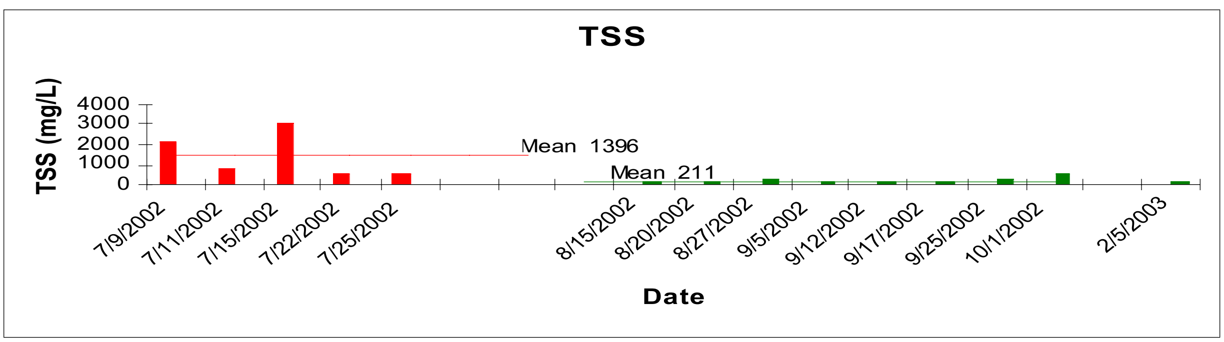
Following pumpout and hydrojetting of grease trap lines on 8/06/2002, a BioAmp® was placed on-line and testing continued from 8/15/2002 to 2/05/2003.

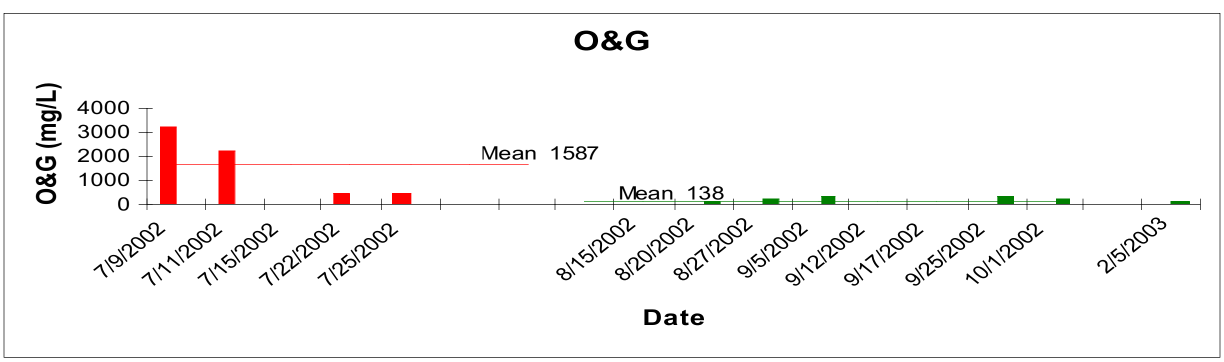
All effluent level means showed significant and consistent reductions as evidenced by the green bars for each test.

Figure 4 - Food Retail Services for grease trap









DISCUSSION

The challenge of developing a system that is cost effective and simple to operate, but also dispenses high levels ($3x10^{13}$/day) of selected strains of *Pseudomonas* and *Bacillus* species on site has been accomplished.

The efficacy data presented demonstrates that the BioAmp® is capable of reducing FOG, BOD, COD and TSS in commercial businesses that employ grease traps for waste control. This reduction results in fewer pumpouts and plumbing calls during the year, reducing customer cost. In addition, the mixed food industrial data clearly demonstrates that BOD reduction is achieved. The efficacy data presented shows a mean BOD reduction of 41.6% with a standard deviation reduction of 64.6% over a very short retention time (1-3 hours). Development of this information resulted in a significant reduction in surcharges for the customer.

Additional validating data was evident by the fact that the BOD returned to prior levels when the BioAmp® units were shut off. This was done at the customer's request to determine if the system really worked. The results speak for themselves, when the BioAmp® units were turned back on, their BOD levels went down. Added benefits were demonstrated at the receiving waste treatment municipality 18 miles away from the mixed food facility. This waste treatment facility utilizes a trickling filter system designed for 80% to 90% ammonia removal. During the past five years, average ammonia removal has been 20% to 30%. In the first month following start-up of the BioAmp® units, an 80% ammonia removal was achieved. When the BioAmp® units were turned off, the ammonia removal levels returned to 20% to 30%. When the units were turned back on, the ammonia removal returned to the 80% to 90% levels.

CONCLUSIONS

Based on aforementioned results, substantial benefit can be attained from the implementation of the BioAmp® system in process water collection and treatment systems, lift stations, grease traps, etc. The BioAmp® automatically generates 30 trillion bacteria daily on site from only 3.2 grams of bacterial inoculum. Unlike traditional dry mix bacterial products which requires 6 hours to emerge from dormancy, this system generates live vegetative bacteria ready to consume BOD and TSS upon entering the waste stream.

This system is automated, compact, easy to operate and install, and requires only tap water and 110V outlet.

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