Field Experience with an Integrated Biological Degreasing System

Timothy P. Callahan and Mikael Norman BioClean USA, LLC, Bridgeport, CT Juan Hajdu Haydu Associates, Orange, CT

While microorganisms have been used for many years to digest oil from wastes and spills, the integration of biodegradation with aqueous cleaning for metal finishing applications is a relatively recent process. Most of the conventional cleaners will not allow the survival of oil consuming microbes due to high operating pH and temperatures. By formulating a mild alkaline emulsifying cleaner that operates at relatively low temperatures it is possible to integrate the removal of oil and particulates with the biological digestion of the residues. The system is essentially self-regulating, since the microbial activity will adjust itself to the amount of oil present in the system

The paper reviews the mechanism of biological degreasing and the long term field experience with a system applied to surface finishing industries, such as anodizing, zinc, nickel and brass electroplating, conversion coatings, painting and powder coating.

For more information, contact Timothy P. Callahan BioClean USA 40 Cowles Street Bridgeport, CT 06607

 Telephone:
 (203) 367-0663

 FAX:
 (203) 367-0396

 E-mail
 biocleanus@aol.com

INTRODUCTION

While microorganisms have been used for many years to digest oil from wastes and spills, the integration of biodegradation with aqueous cleaning for metal finishing applications is a relatively recent process. Most of the conventional cleaners will not allow the survival of oil consuming microbes due to high operating pH and temperatures. By formulating a mild alkaline emulsifying cleaner that operates at relatively low temperatures it is possible to integrate the removal of oil and particulates with the biological digestion of the residues. The system is essentially self-regulating, since the microbial activity will adjust itself to the amount of oil present in the system.

The BioClean system employs an alkaline cleaning solution and control system that utilizes microbes in the solution to consume the oil/grease that is removed from parts during the cleaning process in the metal finishing industry. The system operates at relatively low temperatures (104°F – 131°F) (40°C - 55°C) and a pH range of 8.8 - 9.2, which is a viable habitat for these microorganisms. The cleaning process actually takes place in two separate operations. When parts come in contact with the solution, the oil and impurities are emulsified into micro-particulates. The particulates are then consumed by microorganisms, which are present in the bath or spray. The microbe consumption of the oil present in the bath, as its food source, results in the production of CO₂ as a by-product. The microbes are naturally present in industrial oils and greases, and the main species responsible for biodegradation has been identified as pseudomonas stutzeri, a microorganism found in soil and water.

The cleaning solutions from the cleaning baths are pumped continuously into a separator module that feeds the system. After treatment the cleaning solution is returned into the holding tank and then pumped back into the cleaner tanks. This operation is run in a continuous mode without interruptions for solution dumping and new solution make-up. As a result of the dynamics of the process and the re-circulation of the bath solution, the consumption of oil by the microbes occurs throughout biological degreasing system. For an efficient operation the oil must be emulsified and oil must be present at all time to keep an active population of microorganisms. In the case of a longer interruption that may be conducive to the total depletion of the oil present in the system, to keep the microbes alive it is necessary to render them dormant by increasing the pH to 10.5 or alternatively, to feed them with small amounts of oil during the down time.

The system is managed by a separator control module (Fig. 1), consisting of a process tank, inclined plate clarifier, aerator, heaters and heat control, pH meter, transfer pump, chemical metering pumps. The module controls the temperature and the pH, and the replenishment of surfactants and nutrients, maintaining the chemical and biological equilibrium. It is possible to operate the system without downtime for extended periods (up to many years), eliminating the need of dumping spent cleaning solutions. The enhanced productivity and the reduced use of chemicals and water have made the system well suited to fulfill the present needs of the industry.

The biological cleaning system can be adapted to the requirements of a broad range of industrial applications, and currently the process is used in electroplating, painting, powder coating anodizing and general metal working operations. The objective of this paper to examine the principles of the biological degreasing process and to review a number of case histories, illustrating the flexibility and adaptability of this new approach to industrial cleaning.



Figure 1: Biological Cleaning Control Module

PRINCIPLES OF BIOREMEDIATION

The consumption of emulsified oil in the cleaning process by microorgamisms is essentially bioremediation. In the simplest terms, bioremediation is the use of microorganisms (fungi or bacteria) to decompose pollutants into less harmful compounds. Bioremediation is the technological application of biodegradation. Biodegradation is a natural process by which microbes alter and break down petroleum hydrocarbons, natural oils and fats into other substances. The resulting products can be carbon dioxide, water, and partially oxidized biologically inert by-products¹. Bacteria that consume petroleum are known as "hydrocarbon oxidizers: because they oxidize compounds to bring about degradation².

Bioremediation is the optimization of biodegradation. This acceleration can be accomplished by two forms of technology: (1) fertilizing (adding nutrients) and/or (2) seeding (adding microbes). These additions are necessary to overcome certain environmental factors that may limit or prevent biodegradation. Certain enzymes produced by microbe's attack hydrocarbon molecules, such as oil, causing degradation. The degradation of oil relies on having sufficient microbes to degrade the oil through the microbes' metabolic pathways (series of steps by which degradation occurs).

Fortunately, nature has evolved many microbes to do this job. Throughout the world there are over 70 genera of microbes that are known to degrade hydrocarbons³, which account for only 1% of the natural populations of microbes. The bacteria utilized by the Biological Cleaning process are *pseudomonas stutzeri*. However, even when these microbes are present, degradation of hydrocarbons can take place only if all other basic requirements of the microbes are met.

Nutritional Requirements

Bacteria differ dramatically with respect to the conditions that allow their optimal growth. In terms of nutritional needs, all cells require carbon, nitrogen, phosphorus, sulfur, numerous inorganic salts (potassium, magnesium, sodium, calcium, and iron), and a large number of other elements called micronutirients. The survival of a microorganism depends on whether or not it can meet its nutritional needs.

Carbon is the most basic structural element of all living forms and is needed in greater quantities than other elements. The nutritional requirement ratio of carbon to nitrogen is 10:1, and carbon to phosphorus is 30:1⁴. Organic carbon is a source of energy for microbes because it has high energy yielding bonds in many compounds. In the decomposition of oil, there is plenty of carbon for the microorganism due to the structure of the oil molecule.

Nitrogen is found in the proteins, enzymes, cell wall components, and nucleic acids of microorganisms and is essential for microbial metabolism. Because only a few microorganisms can use molecular nitrogen, most microorganisms require fixed forms of nitrogen, such as organic amino nitrogen, ammonium ions, or nitrate ions⁵. These other forms of nitrogen can be scarce in certain environments, causing nitrogen to become a limiting factor in the growth of microbial populations.

Phosphorous is needed in the membranes (composed of phospholipids), ATP (energy source of cell) and to link together nucleic acids.

Environmental Requirements for Microbial Growth

Along with nutrients, microbes need certain conditions to live. Microbial growth and enzymatic activity are affected by stress ultimately impacting the rate of biodegradation. As the stress increases (less favorable conditions occur) the microbes have a harder time living in their environment. Just as humans need certain conditions to live (like oxygen) so do microbes. There is a certain range of conditions in which microbes can live. As conditions reach the extremes microbial growth slows down, but when conditions are perfect the microbial community can thrive.

Oxygen is needed, since biodegradation is predominantly an oxidation process known as heterotrophic metabolism. Bacteria enzymes will catalyze the insertion of oxygen into the hydrocarbon so that the molecule can subsequently be consumed by cellular metabolism¹. Because of this, oxygen is one of the most important requirements for the biodegradation of oil. The primary source of oxygen for biodegradation is atmospheric oxygen. Aeration is required to allow biodegradation to take place. Oxygen is important in hydrocarbon degradation because the major pathways for both saturated and aromatic hydrocarbons involve molecular oxygen or oxygenases⁴. Theoretical calculations show that 3.5 g of oil can be oxidized for every gram of oxygen present.

Biodegradation can also occur under anaerobic conditions by processes called anaerobic respiration, in which the final electron acceptor is some other inorganic compound, such as nitrates, nitrites, sulfates, or carbon dioxide. The energy yields available to the cell using these acceptors are lower than in respiration with oxygen – much lower in the case of sulfate and carbon dioxide – but they are still substantially higher than from fermentation..

Water is needed by microorganisms since it makes up a large proportion of the cell's cytoplasm. Water is also important because most enzymatic reactions take place in solution. Water is also needed for transport of most materials into and out of the cell.

Several variables, including pressure, concentration temperature and pH, may also have important effects on biodegradation rates. Bacteria have adapted to a wide range of temperatures. Although hydrocarbon degradation has been found to occur at a wide range of temperatures (as low as below 0° C to as high as 70° C) it is an important factor on the rate of biodegradation.

Raising the temperature will increase the possibility of reactions taking place and increase the rate of diffusion. Without reactions and diffusion life cannot exist. In general the rate of enzymatic reactions can be doubled for every 10°C rise in temperature as long as the enzymes are not denatured⁵. The higher the rate of the enzymatic reactions the faster the biodegradation will occur. However, there is a maximum temperature at which these microorganisms successfully survive. While higher temperatures are conducive to cleaning, temperatures in excess of 60°C will kill the bacteria. For this reason the temperatures for Biological Cleaning are maintained between 40° and 57°C (104-131°F).

In the Biological Cleaning process the pH of the cleaner is also an important variable and it is maintained in a relatively narrow range of 8.8 to 9.2. At pH values above this limit the microbial activity decreases, while at lower pH values the microbe population will grow too fast and will consume not only the oils present but also the biodegradable surfactant needed for cleaning.

The concentration of pollutants is an important factor. If the concentration of petroleum hydrocarbons is too high then it will reduce the amount of oxygen, water and nutrients that are available to the microbes. This will create an environment where the microbes are stressed thereby reducing their ability to break down the oil.

Once the necessary requirements are present either naturally or by addition, the oil can begin to be broken down by the microbes. Favorable conditions for the microbes will help optimize the degradation of the oil. The degradation of these hydrocarbons occurs in certain steps and can be represented by metabolic pathways.

Metabolic Pathways for Oil Decomposition .

There is a multitude of oils. The difference in composition determines the quality of any particular oil. Petroleum is a complex mixture of hydrocarbons, but it can be fractionated into aromatics, aliphatics, asphaltics and a small portion of non-hydrocarbon compounds. Over the last 20 years complex chemical equations have been derived to describe the metabolic pathways in which oil is broken down. The general outline bioremediation pathways for aliphatic and aromatic hydrocarbons have been formulated and continue to be developed in greater detail with time⁶. All of these pathways will result in the oxidation of at least part of the original hydrocarbon molecule¹. The content of a particular petroleum mixture will also influence how each hydrocarbon will degrade⁴ and the type and size of each hydrocarbon molecule will determine the susceptibility to biodegradation 7 .

Aliphatic hydrocarbons_also known as the saturates include compounds such as n-paraffins, iso-paraffins and alicyclic hydrocarbons (cycloparaffins). The type and size of the hydrocarbon molecule will affect its ability to be metabolized by microorganism⁷. The straight-chain alkane (n-paraffin) compounds with 10 to 24 carbon

atoms are degraded the fastest because they are easiest to metabolize³. The shorter chains are toxic for many microorganisms⁴. As the length of a chain increases, it becomes resistant to biodegradation, and those compounds with molecular weights of 500 to 600 are no longer able to serve as a carbon source due to its length⁴. Branching of alkanes will reduce the biodegradability³. For more detailed information on the order of degradation see Atlas and Bartha "Microbial Ecology"⁷.

Aromatic hydrocarbons are made up of at least one benzene ring or substituted benzene ring. These compounds can be degradable when they are simple and have a low molecular weight. However, as they increase in complexity and molecular weight they are not as easily degraded³. Aromatics with five or more rings are not easily attacked and may persist in the environment for long periods of time.

Asphaltenes are difficult to describe with current methodology because of their complexity, these compounds are not well understood⁴. No uniform degradative pathway, comparable to the pathways established for aliphatic and aromatic hydrocarbons, has yet emerged for the asphaltic petroleum components and these compounds do not or are slow to biodegrade³.

Recently further developments in microbial biodegradation of pollutants were announced by an international team of scientists from the University of Connecticut, Jeonju University (South Korea) and the University of Milan. The bacterium, *Pseudomonas stutzeri OX1*, exuded an enzyme that degrades tetrachloroethylene (PCE) in the presence of oxygen, converting the chemical into harmless chloride ions⁸. A different strain of the same bacterium. Pseudomonas stutzeri KC, is one of the organisms few that can degrade carbon tetrachloride⁹.

Additionally, studies have demonstrated that additional strains of the same bacterium degrade a unique set of potential pollutants (*pseudomonas stuzeri KS25* can aerobically degrade 2chlorobenzoate, 2,3-dichlorobenzoate, 2fluorobenzoate, 2-iodobenzoate, 2-bromobenzoate and 2,5-dihydroxybenzoate; *pseudomonas stuzeri OM1* can aerobically degrade Carbazole; *pseudomonas stuzeri OX1* can aerobically degrade toluene and xylene).

FIELD EXPERIENCE

Several installations were selected to illustrate the field performance of the Biological Cleaning systems in various metal finishing applications. The operations selected have been in service continuously for extended periods (from one to four years) representing the long term experience with this approach to oil and grease removal and elimination.

Metal Working.

More than three years ago Hubbell Incorporated, a large manufacturer of electrical components and wiring devices located on the East Coast of the U.S. was confronted with the need to change their cleaning system based on methylene chloride to an more environmentally friendly process. The decision was made to change to a water based system, but this alternative presented several problems. It required a relatively expensive installation (estimated at \$300,000) to treat the spent cleaner to de-emulsify the oil. The plant used a septic system and was not connected to sewers, all spent materials would have required to be hauled away for waste treatment. Also, the equipment used for solvent cleaning could not be adapted for The additional floor space aqueous cleaning. required for alkaline cleaning was another reason why management was willing to consider other approaches to the problem.

The biological cleaning process offered a very attractive alternative to conventional alkaline cleaning. After more than three years of continuous use, the company's management considers biological cleaning a very robust process; simple to maintain and economically sound. The cleaner handles carbon steel, stainless steel and zinc, brass and nickel plated parts. The original solution is still in the system, maintained by automatic controls and replenishment. The control unit requires only one hour of maintenance per week, spent mostly in checking and replacing the containers of the replenishing materials. The only residue of the process is the small amount of dead bacteria removed from the control unit, which, being completely biodegradable, is disposed by the plant's septic system.

One of the main advantages of using the biological cleaning system was the possibility of adapting the existing in line solvent cleaning equipment to biological cleaning. Today the original cleaning equipment modified by the plant's maintenance personnel is used with excellent results. Since the cosmetic appearance of the parts is of a critical importance, a warm deionized water rinse was introduced in the washer, which has eliminated all water stains. A portion of the rinse water is used to maintain the level of the cleaner. while an evaporative unit handles the rest of the rinse. The system is essentially a zero discharge system. In an other plant of the company, located in the Commonwealth of Puerto Rico, that manufactures parts used in assembled products that do not require the same level of cosmetic appearance, rinsing is not used. The small amount of wetter left on the surface acts as a protective finish

An interesting aspect of the biological cleaning system is the flexibility of the microorganisms in adapting their activity to digest a large variety of oils. In this installation carbon steel stampings represent the main workload and partially chlorinated oil (Fuchs Tuffdraw), is used as a stamping lubricant. Tests indicate that more than 99.8% of the contamination is consistently removed from the surface of the stampings by the biological cleaner and that the bacteria continuously consume the emulsified oil. Oil is added to the system at the rate of 2,800 g per 8-hour shift, processing an average of 250 sq. meters (3,000 sq. ft.) of work per shift.

An other interesting example of metal cleaning after stamping can be observed at Allied Metals Corporation, Detroit, MI. This company collects, cleans and recycles secondary metals generated during the stamping of steel parts. They handle from 8,000 to 12,000 tons of steel a month and which is washed to remove the lubricants used in stamping. The cleaning is done in a rotary screw machine and the biological cleaner is applied by spray. The steel is then rinsed and dried and the total operation lasts from five to eight minutes, and the average de-oiling is done in two minutes. The total production is handled in one shift. Since the steel is heavily contaminated with dirt and other residues, the biological degreaser has to handle general cleaning in addition to removing oil, and a settling tank was added to the installation to remove these residues.

The total volume of the cleaner is 3,000 gals and after two years, the original solution is still in The rotary washer causes a strong cross use. contamination between the cleaning step and the rinse. For this reason it became necessary to feed back the rinses into the biological digestion module. To make room for the addition of the rinse waters. part of the cleaning solution is continuously evaporated in an atmospheric evaporator. The steam from the evaporator is condensed and reused as a rinse, and the system is operating almost as a closed loop system. The only loss of water is caused by evaporation in the drying step. Before installing the Biological Cleaner, conventional chemical cleaning was used and the cleaners had to be dumped two to three times a week. Changing over to biological cleaning has resulted in major economies of chemicals, labor and water.

Anodizing

Florida Anodizing & Coloring, Inc., a small finishing shop in Bradenton, FL, has been using biological cleaner during the last three years. This process has replaced the alkaline cleaners used previously. The alkaline cleaning solutions lasted about three months, after which they had to be disposed through conventional waste treatment. As the cleaning solutions aged, they became contaminated with oil, which was carried over into the etch and the anodizing tanks. Also the former oil removal process was slower in the older cleaners, requiring longer soaking times, reducing the productivity of the line.

The biological cleaner has solved this problem. It operates essentially in a steady state mode avoiding the build up of oil in the cleaning tank. The composition of the cleaning solution is consistent and for this reason the processing times are constant and independent from the age of the bath. Avoiding the contamination with oil has also extended the life of the etches. The improved productivity and absence of down time has allowed significant cost savings. The savings from eliminating the dumping and treating the spent cleaners and etches is estimated at \$12,000 and the savings in chemicals at \$4000 per year.

In addition to the savings in materials and waste treatment, significant quality improvements were obtained. Some of the parts anodized had precision threading coated with hydraulic oil, which could go out of tolerance during the extended soak cleaning and etching processes, especially on rejects that had to be reworked using the conventional process. The biological cleaner by reducing the soak cleaning times from 20 minutes to 3 minutes and the etching times from 3 minutes to 15 seconds has allowed for much milder conditions, preventing the attack of the parts during the process.

Painting and Powder Coating

Eastern Process Company, Hingham, MA specializes in painting and powder coating medium and large size sheet metal parts. The substrate is mainly steel, with aluminum and galvanized steel parts intermixed with the work. All parts are treated in a five-stage spray washer, where a rinse, iron phosphate, final rinse and hot air drying follow the Biological Cleaner. The Biological Cleaner was installed approximately three years ago and the original solution is still used. Replenishment and control of the cleaner is handled automatically by the control equipment. The washer handles up to 1500 sq. ft. per hour, in a 1600 gal. Biological cleaning system. In this installation the biological cleaning replaced a warm alkaline spray cleaner that was only partially successful in removing the oils. In many cases it was necessary to hand clean larger parts prior painting. The present system has significantly reduced the number of rejects and reduced the operating cost by saving chemicals and labor.

An interesting aspect of this operation is the flexibility demonstrated by the biological cleaning process. Due to the mechanical conditions in the spray washer, the cleaner is contaminated by the phosphating solution. By selecting compatible products, this cross contamination has not affected the biodegradation process and the cleaning solution is performing essentially in a steady state mode.

Electroless Plating

This large job plater is located in Eastern US and specializes in high quality electroless nickel plating of electronic components. Here biological cleaning has replaced solvent degreasing that was used to clean steel parts coated with several different types of lubricant, such as animal fats, electrostatic oils and synthetics. All the work is handled in barrels and the rest of the cycle is the same as used after solvent degreasing. They are using a hot alkaline soak cleaner and an electrocleaner after the biological degreaser.

The biological cleaner has been in use for over one year, and compared with other aqueous degreasers the main benefit noted has been consistency. The other solvent free approaches to degreasing that were tried started out by being effective, but as the lubricant content increased the cleaner would loose its degreasing activity. Frequent dumping was required and consistent quality was hard to attain. Electroless nickel coatings can easily peel or blister and the biological degreaser has increased the reliability of the operation.

Electroplating

Two different electroplating operations will be briefly described. National Manufacturing Company has two zinc plating operations, one in Rock Falls IL and a second operation in Sterling, IL. Both are captive plants, plating mainly fasteners and architectural hardware in rack and barrel. The cleaning operation at Sterling has been studied in detail and reported by the Concurrent Technologies Corporation under a Cooperative Agreement with the U.S. Environmental Protection Agency. This report will be presented under the title "Evaluation of BioClean USA, LLC Biological Degreasing System for the Recycling of Alkaline Cleaners" during the 22nd Annual AESF/EPA Conference on Pollution Prevention and Control for the Surface Finishing Industry, January 29-31, 2001, in Orlando, FL.

The installation In Sterling contains four plating lines, three barrel lines and one rack and barrel. The base metals are cold rolled steel, zinc dye castings, stainless steel and brass. The cleaning cycle includes the following steps: biological cleaning, electrocleaning, rinse, rinse, acid, rinse, rinse and at the time of the evaluation, the degreasing solution was over twelve months old. The cleaning solution is continuously pumped to holding tank that feeds the control unit. The biodigestion of the oil occurs in the cleaning tanks, the holding tank and the control unit.

Comparing the cost of biological cleaning with conventional alkaline cleaners used formerly, the total annual savings are approximately \$80,000. The major contribution to these savings comes from lower chemical usage (\$67,300), and reduced energy, labor, and waste disposal costs. Projecting the savings to a second and a third year of use would allow increasing economies by further extending the life of the biological cleaning solution.

The second example of biological cleaning used in en electroplating operation reviews the experience at Jagemann Plating Company, Manitowoc, WI. This company has originally received a grant from the State of Wisconsin to test Biological Cleaning and the results of the test are available to any person interested in the subject by contacting Mike Jagemann, Executive Vice President (920) 682-6883.

This company does job metal finishing and electrodeposits zinc in barrel and rack operations, bright nickel and chrome rack plating and barrel copper and nickel plating. They plate a large variety of steel parts, which are covered with many types of oils and greases. All parts are degreased by Biological Cleaning, which was installed two years ago. The cleaning solution installed initially is still being used at the present operating twenty-four hours, seven days a week. The Biological Cleaner is maintained at 40°C (105°) with immersion times between one and five minutes, depending on the condition of the work. Most parts are completely degreased in two minutes.

Originally chemical cleaners were used at Jagemann Plating and in spite of running them at high temperatures (70°C, 160°F) they presented many problems of incomplete degreasing. The alkaline cleaning tanks had to be dumped frequently (about nine times a year) generating waste and requiring labor. The economies in labor, make up and replenishment chemicals and waste reduction has resulted in annual savings of approximately \$23,000. This number does not include the savings resulting from lower water usage (estimated at 10,000 gallons a year) and the decrease in gas usage due to the lower operating temperature of the Biological Cleaner.

CONCLUSIONS

Four years of field experience has established Biological Cleaning as a valid approach to oil and grease removal and elimination in a wide variety of metal finishing operations. The main advantages of the process are both environmental and operational. Using this approach it is possible to extend the life of the degreasing solution, eliminating the need of disposal of liquid and solid wastes. Biological Cleaning allows for major savings in the consumption of water, energy and chemicals, and reduces the general maintenance costs of the cleaning operation.

In addition to the environmental and operational benefits, Biological Cleaning has a major quality advantage over conventional aqueous cleaning. The microorganisms present in the system consume the oils and greases removed from the parts, preventing the build-up of contaminants in the cleaning solution. The solution is continuously rejuvenated and process runs essentially in a steady state mode, a condition that is conducive to consistent operating parameters and high yields. By integrating the cleaners with automatic control equipment, it is possible to create a process that is robust, economical and reliable.

REFERENCES

¹ Bragg, J.R.; Prince, R.C.; Wilkinson J.B.; Atlas, R.M. Bioremediation for Shoreline Cleanup Following the 1989 Alaskan Oil Spill. Houston, TX: Exxon Co.; 1992.

² Chanelli, R.R; et al. Bioremediation technology Development and Application to the Alaskan Spill. International Oil Spill Conference; 1991 march 4-7; San Diego, California: U.S. Coast guard, American Petroleum Institute, and U.S. Environmental Protection Agency: 1991: 549-558.

³ U.S. Congress, Office of Technology Assessment, Bioremediation far Marine Oil Spills - Background Paper. 1991a May. 31p. Available from: U.S. Government Printing Office. Washington DC: OTA-RP-O-70.

⁴ Atlas, R.M.; Bartha, R. Microbial Ecology: Fundamentals and Applications. Reading, Ma: Addison-Wesley publishing Company; 1981.

⁵ Atlas, R.M. Microbiology: Fundamentals and Applications. New York: Macmillian Publishing Company; 1984.

⁶ Glaser, J.A.; Venosa, A.D.; Opatken, E.J. Development and Evaluation of Application Techniques for Delivery of Nutrients to Contaminated Shoreline in Prince William Sound. International Oil Spill Conference; 1991 March 4-7; San Diego, California: U.S. Coast Guard, American Petroleum Institute, and U.S. Environmental Protection Agency: 1991: 559-562.

⁷ Atlas, R.M.; Bartha, R. Microbial Ecology: Fundamentals and Applications. 3rd ed. Reading, Ma: Addison-Wesley publishing Company; 1993.

⁸ Ryoo, D.; Hojae, S.; Canada, K./ Barbieri, P.; Wood, T. Aerobic Degradation of Tetrachloroethylene by Toluene-o-xylene monooxygenase of Pseudomonas Stutzeri OX1. Nature Biotechnology. 18: 775-778, 2000.
 ⁹ Worden, R.M.; Ngo, B. The Chemotactic Movement of Pseudomonas Stutzeri KC. Department of Chemical Engineering, Michigan

State University; 1999 August 22