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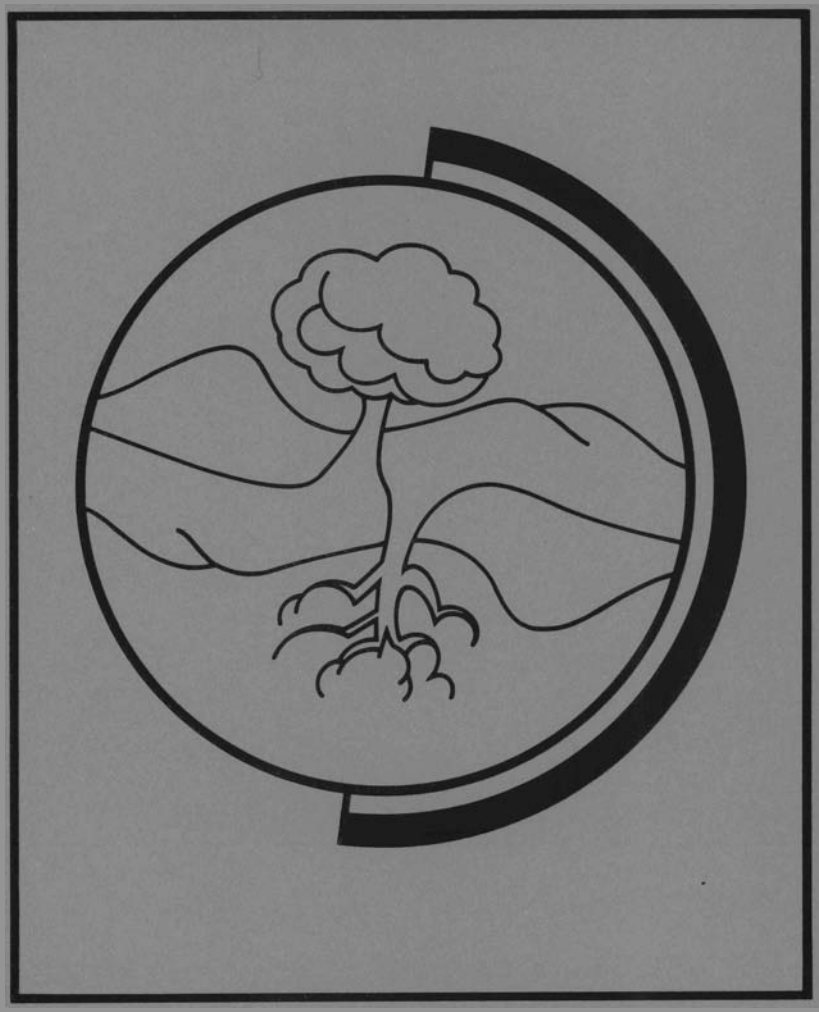
Environmental Assessment of the Potential Effects of Aquifer Thermal Energy Storage Systems on Microorganisms in Groundwater

March 1988



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ENVIRONMENTAL ASSESSMENT OF THE POTENTIAL EFFECTS OF AQUIFER THERMAL ENERGY STORAGE SYSTEMS ON MICROORGANISMS IN GROUNDWATER

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March 1988

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SUMMARY

The primary objective of this study was to evaluate the potential environmental effects (both adverse and beneficial) of aquifer thermal energy storage (ATES) technology pertaining to microbial communities indigenous to subsurface environments (i.e., aquifers) and the propagation, movement, and potential release of pathogenic microorganisms (specifically, *Legionella*) within ATES systems. Seasonal storage of thermal energy in aquifers shows great promise to reduce peak demand; reduce electric utility load problems; contribute to establishing favorable economics for district heating and cooling systems; and reduce pollution from extraction, refining, and combustion of fossil fuels. However, concerns that the widespread implementation of this technology may have adverse effects on biological systems indigenous to aquifers, as well as help to propagate and release pathogenic organisms that enter these environments need to be resolved.

Groundwaters are habitat to an abundant and diverse microbial population. The activities of these organisms are important to the removal of a variety of hazardous wastes. However, these organisms have only recently been studied, and their full importance is not known at this time. The growth and activity of microorganisms indigenous to aquifers are governed by a variety of environmental factors including:

- the porosity of the aquifer
- nutrient availability
  oxidation-reduction conditions
- pH
- temperature
- the adsorption of microorganisms to subsurface particles.

Perturbations, due to the implementation of ATES technology, can locally alter these physicochemical factors which can, to an unknown degree, affect the growth and activities of microorganisms inhabiting aquifers.

Of the environmental factors listed above, groundwater temperature alterations caused by injection of heated or cooled waters during the operation of

ATES systems have the greatest potential for altering the types and activities of native microbial communities. Microorganisms, depending on the species, have specific temperature ranges that allow for their growth. If the use of ATES systems alters the temperature of an aquifer beyond the range that supports growth, microbial activity will become dormant or cease. Other ATES-related alterations that can cause changes in the microbial ecology of aquifers include groundwater chemistry (e.g., pH, Eh, solubility of minerals) and changes in physical characteristics (e.g., adsorptive to subsurface particles, hydraulic conductivity). However, the true impact of such changes in the microbial ecology and activity can not yet be assessed. Experience with ATES in other countries, as well as in the U.S., has not indicated major impacts; however, intensive monitoring programs have not been conducted in conjunction with the operation of most systems.

In addition to indigenous microbial populations, allochthonous organisms (i.e., organisms foreign to that environment) can survive and grow in aquifers. Allochthonous microorganisms detrimental to human health (i.e., pathogens) are of particular concern. The effect of ATES technology on the survival, propagation, and release of Legionella was a major focus of this document because of the ubiquity of Legionella in aquatic environments and its pathogenicity. Of the ATES systems currently in operation, chill systems appear to have the highest potential for increasing *Legionella* survival (as well as the survival of other pathogens). Propagation of these organisms is often associated with systems connected to open-cycle chill ATES systems (e.g., cooling towers operated with groundwater cooled by ambient air) and pathogen survival, in general, is increased with decreasing temperature.

The capacity of ATES, and particularly chill ATES, to augment transport of pathogenic organisms, such as Legionella, must be kept in perspective. Groundwater is widely used in systems other than ATES (such as irrigation and groundwater heat pumps) and would be expected to cause largely the same effects on the geomicrobial ecology and surface transport of related microorganisms. Yet these practices have not been associated with significant microbial changes or negative impacts. Furthermore, the aquifer storage zone is normally very

small, rarely encompassing a projected area larger than 112-acre for a commercial building. Thus, it is expected that groundwater microbial changes will normally be confined to a small zone within the aquifer. Finally, an open-cycle ATES system wherein groundwater is in direct contact with the atmosphere has greater potential for above-ground microbial transport than the commonly-proposed closed-cycle (i.e., no direct contact) ATES systems.

Aquifer thermal energy storage technology has the potential to affect the survival, growth, and activities of microorganisms inhabiting aquifers. The specific changes that occur, their relative importance versus the beneficial aspects of ATES technology, as well as methods for controlling adverse effects are areas of study recommended in this document.

ACKNOWLEDGMENTS

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GLOSSARY

Actinomycetes - Microorganisms that have both bacterial and fungal characteristics. Some have branched filaments with cubed terminal bodies, which are fragmented into bacteria-like cells. Others form sporulating mycelia.

Allochthonous - Microorganisms not considered natural inhabitants of the environment in which they are found (i.e., foreign to the environment).

Amoeba - A type of protozoa characterized as having an indefinite changeable form and moving by means of pseudopodia.

Anaerobic - Capable of living in the absence of oxygen.

Anthropogenic - Man-made or derived.

Chemoheterotrophs - Microorganisms that require organic carbon as an energy and carbon source.

Ciliates - Unicellular organisms that contain special, hair-1ike locomotor organelles that extend out from the surface of the cell.

Culturability . The ability to grow microrganisms in a nutrient medium.

Denitrifying - Having the ability to reduce nitrate to nitrogen.

Dissimulatory capacity - An organism's ability to degrade a variety of compounds.

Embryonated - Fertilized.

Enteric viruses - Viruses associated with feces-contaminated food and water.

Exogenous - Derived or developed from external causes.

Fermentative - The use of organic compounds either as electron donors or acceptors.

Fungus - Any of numerous plants of the division and subkingdom Thallophyta, lacking chlorophyll, ranging in form from a single cell to a body of mass-branched filamentous hyphae that often produce specialized fruiting bodies.

Heterotrophic - Requiring organic carbon for metabolism.

Hydrogen oxidizers - Autotrophic hydrogen bacteria that possess the enzyme hydrogenase, which allows them to employ the oxidation of molecular hydrogen as the source of energy for fixation of carbon dioxide and all other vital processes.

Hydrophobic - Incapable of dissolving in water.

Indigenous - Occurring or living naturally in an area; not introduced, native.

Mesophilic - Capable of growing at moderate temperatures ranging from 25 to 37°C.

Metabolites - Any of various organic compounds produced by metabolism of microorganisms.

Methanogenic - Producing methane under strict anaerobic conditions.

Microflora - Composite of microorganisms.

Motility - Movement of microorganisms through a medium.

Myalgia - Muscular pain.

Nitrifying bacteria - Aerobic and autotrophic bacteria capable of oxidizing ammonia to nitrite and/or nitrite to nitrate.

Nitrogen fixers - Microorganisms that fix nitrogen gas into ammonia, which is used for the synthesis of cellular N compounds.

Oligotrophic - Limited organic nutrients available, usually less than 0.1 ppm.

Oxygenase enzymes - Enzymes that catalyze the oxidation of a substrate.

Pathogenic - Capable of producing disease.

Pheophytin - A bluish-black waxy pigment that is olive brown in solution and that is obtained from chlorophyll by mild acid treatment.

Plasmids - Small, self-replicating molecules of DNA that are separate from the main chromosome DNA in bacteria, yeasts, and plants.

Prokaryotic - Having no nucleus in the cell; DNA is not enclosed within a membrane.

Protozoa - Single-cell organisms that are classified according to the type of motility they exhibit (i.e., ameboid activity, cilia, flagella, dissemination of spores).

Quinoline - An aromatic organic base (C_9H_7N) synthesized or obtained from coal tar and used as a food preservative and in making antiseptics and dyes.

Redox - An oxidation-reduction process in chemical reaction.

Septic shock - A response that occcurs in patients who have pathogenic organisms or toxins in their bloodstreams.

Serological - Pertaining to serum.

Spp. - Species.

Substrate - A surface of a material to which microorganims may attach and grow, or the material upon which an enzyme acts.

Sulfur oxidizers - Microorganisms that are able to convert elemental sulfur back to sulfate.

Thermophilic - Capable of growing at optimum temperatures of 55 to 65°C.

Trophic level - Feeding stratum in a food chain of an ecosystem characterized by organisms that occupy a similiar functional position in the ecosystems.

ENVIRONMENTAL ASSESSMENT OF THE POTENTIAL EFFECTS OF AQUIFER THERMAL ENERGY STORAGE SYSTEMS ON MICROORGANISMS IN GROUNDWATER

1.0 INTRODUCTION

The acceptability of aquifer thermal energy storage (ATES) technology depends, in part, on the impact aquifer storage systems have on the environment. That is, the sum total of positive effects must outweigh the sum of the negative effects, and no one negative environmental effect may be intolerable (Reilly 1980). Previous assessments of ATES systems indicate that these technologies will have a net positive effect on the environment, primarily due to a reduced national demand for fossil fuels and the resultant reduction in pollution from the extraction, refining, and combustion of those fuels (Reilly 1980). Some potentially negative effects, such as thermal pollution of aquifers, chemical contamination, changes in regional groundwater flow, and stimulation of seismic activity, have been evaluated (Reilly 1980) and deemed to be unimportant or avoidable with proper engineering design, careful operation, and continuous monitoring. However, the effect ATES systems may have on aquifer biological systems, including changes in the indigenous microbial populations or on the propagation and release of pathogenic microorganisms, has not been examined.

The purpose of this review is to evaluate the potential influence (both adverse and beneficial) ATES systems may have on microbial populations in groundwaters (indigenous and allochthonous), to identify gaps in the present understanding of the effects of ATES technology on subsurface microorganisms, and to suggest research directions that may remedy current limitations to this understanding.

1.1 SCOPE OF REVIEW

A summary of the conclusions drawn from the literature review and recommendations for evaluating the effect of ATES systems on microbial populations indigenous and allochthonous to aquifers are provided in Section 2.0.

Section 3.0 provides a brief background of ATES systems and discusses potential microbiological problems that might occur from the widespread implementation of ATES technology. Defining potential problems is necessary before it can be determined whether current knowledge is adequate for evaluating potential effects.

Section 4.0 discusses the subsurface as a habitat for microorganisms, including the environmental parameters most likely to influence microbial activity. In addition, a brief description of the abundance, diversity, and activities of indigenous populations is given, as well as an assessment of the survival and growth of pathogenic organisms in these environments.

Potential environmental impacts of ATES technology on indigenous microorganisms and the survival, growth, and transport of pathogenic microorganisms is discussed in Section 5.0. Particular emphasis is placed on the effect of ATES systems on the propagation and release of *Legionella*. This emphasis is warranted because of the pathogenicity of this bacterium, as well as its common occurrence in waters associated with ATES systems (e.g., cooling towers).

2.0 CONCLUSIONS AND RECOMMENDATIONS

The primary objective of this study was to determine the potential effect (both adverse and beneficial) ATES systems have on microorganisms inhabiting aquifers. The organisms of interest included both those microorganisms indigenous to aquifers, as well as those allochthonous microorganisms that enter aquifers and are able to survive there. The allochthonous microorganisms of particular concern were the *Legionella* bacteria. These organisms are ubiquitous in aquatic environments and can cause serious infections in humans.

Recent studies have shown that aquifers are inhabited by an abundant and diverse indigenous microbial population. Indigenous microorganisms have a major role in governing the behavior of organic contaminants entering aquifers. In addition, numerous pathogenic organisms have been detected within these environments. To determine the potential impact of ATES technology on these microorganisms, it is important to understand the environmental factors that govern their growth and activity and how the implementation of ATES technology affects those factors.

Some of the environmental factors that appear to have a strong effect on microbial activity in groundwaters include

- porosity
- adsorption
- nutrient availability
- redox conditions
- pH
- temperature.

While all of these factors will influence the types and activities of microbiota in aquifers, temperature changes caused by the use of ATES systems have the greatest potential for influencing microbial growth.

Heat systems may prohibit both the indigenous microbiota and pathogenic organisms from entering the aquifer. While the suppression of pathogenic organisms is favored, the loss of important degradative activities of indigenous

microbiota would adversely affect the environmental quality of groundwater by increasing the residence times of contaminants at those locations where contaminants are present. Of course, it must be recognized that ATES would not be sited in a known zone of contamination.

Chill systems do not appear to inhibit the survival of indigenous microorganisms, although their activities would probably be suppressed. An issue of greater importance is the possibility of increased pathogen survival in these systems, especially of *Legionella* spp. These organisms have been associated with cooling towers and cold waters, such as those found in chill ATES systems, and their survival (though not their level of activity) is improved with decreasing temperatures.

If adverse effects arise (i.e., increased pathogen survival and release) following the implementation of ATES technology, it is possible to chemically treat the process waters to destroy the pathogens of interest. However, in doing so it must be recognized that the ecology of the indigenous microbial communities will probably be changed. This imbalance may hinder the ability of these communities to remove organic contaminants entering groundwaters.

To further assess the importance of ATES system to microorganisms in groundwaters, the following recommendations are made:

1. Initial sampling and microbiological assessment of proposed ATES sites should be made to determine the base-line abundance, diversity, and activity of microbial populations within the existing aquifer site.
2. A monitoring program should be developed for ATES pilot test sites to assay changes in the abundance, diversity, and activity of groundwater microbiota. Particular emphasis should be placed on pathogenic organisms (using *Legionella* as a marker organism), and on the biodegradative activities of indigenous microbiota.
3. Research should be conducted to examine the potential use of microbial inhibitors to eliminate pathogenic organisms if increases in pathogenic propagation and release are detected. The effect of the biocides on indigenous microbiota needs to be examined in conjunction with this task.

4. Additional research should be conducted to determine changes in groundwater chemistry caused by the use of ATES systems and the effect of these changes on microorganisms in groundwater.
5. The importance of local impacts (i.e., within the ATES system) relative to overall aquifer quality should be assessed.

3.0 BACKGROUND AND PROBLEM STATEMENT

As a nation, we are challenged with the need to develop alternative energy sources and find ways of using existing energy supplies more efficiently. Seasonal storage and retrieval of thermal energy, using heat or chill available from waste or other sources, shows great promise to reduce peak demand, reduce electric utility load problems, and contribute to establishing favorable economics for district heating and cooling systems.

Storage in aquifers (i.e., ATES) appears to be one of the most economical and widely applicable thermal energy storage techniques. In its simplest form, an ATES installation is composed of a pair of ordinary wells drilled into an aquifer. During operation, the groundwater is withdrawn from one well, heated (or chilled) in a heat exchanger, then injected into the same aquifer through the second well. The thermal energy is stored in the aquifer until needed. At recovery, the second well is pumped, and the hot (or chilled) water circulated through a heat exchanger to recapture the stored energy and then returned to the aquifer through the first well. The thermal energy can then be employed for space or process heating (or cooling), thus reducing the need for generation of primary energy.

Studies have shown that ATES systems are technically feasible and may offer significant savings for utilities, industry, and some commercial operators (Reilly 1980). In a case study involving the Twin Cities District in Minnesota, the potential energy savings of ATES systems was estimated to be greater than 32% over a nonstorage scenario, with a net savings of $31 million (Reilly 1980).

Aquifer thermal energy storage technology also has the potential for reducing environmental impacts from other energy production processes, primarily through reducing the demand for fossil fuels. Reduction in demand for fossil fuels can be translated directly into a reduction in pollutants emitted into the environment and also in a reduction in the capital investment needed to deal with that pollution. In addition to decreasing contaminant emissions, ATES development has the potential for reducing thermal pollution of the atmosphere and surface waters. Approximately two-thirds of the energy content of the

fuel consumed by thermal power plants is discharged to exhaust gases and cooling waters, and then to the environment in the.form of thermal pollution. Cogeneration, made practical by ATES systems, can help to recover much of this lost energy.

Widespread implementation of ATES systems is not without potential problems. The more exotic energy sources present technical problems and research needs to be done to evaluate the implications of widespread heat storage with depth. In addition, numerous legal and financial questions need to be answered. These systems also represent a potential source of thermal and chemical pollution to aquifers. These potential problems have been reviewed by Reilly (1980), and it was concluded that they represent a minor and site-specific aspect. However, an important potential problem that has not been addressed in previous studies, and which is the focus of this report, is the effect of ATES systems on indigenous microbial populations, as well as the potential these systems have for the propagation and release of pathogenic microorganisms, particularly *Legionella.*

Until recently, the terrestrial subsurface was considered devoid of microorganisms. However, within the last 10 to 15 years it has been recognized that subsurface environments are habitat to an abundant and diverse indigenous microbial population. In addition, the large number of outbreaks of disease attributed to microbially contaminated groundwater demonstrates that the subsurface can frequently harbor pathogenic organisms and that the environmental conditions within the subsurface can be conducive to the propagation of these allochthonous populations (Keswick 1984).

It is conceivable that aquifer perturbations caused by the implementation of ATES technology could cause significant imbalances in the microbial ecosystem of groundwaters. These imbalances could influence the biological and chemical quality of groundwater associated with the ATES system. For example, perturbations that adversely affect the ability of indigenous microbial populations to degrade chemical contaminants may cause a lowering in the overall quality of fresh water supplies. Another imbalance that may occur is the reduction of microbial populations antagonistic to pathogen survival and growth. This

reduction may lead to the proliferation of pathogenic organisms within groundwater supplies.

This study was initiated to evaluate the effects of ATES systems on microbial populations indigenous to subsurface environments and to determine the potential for the propagation and release of pathogenic organisms caused by the implementation of ATES systems. The study represents a review of the available groundwater microbiology literature to identify environmental factors controlling microbial activity in the subsurface and to assess the potential for adverse or beneficial effects of ATES systems on those factors. Groundwater microbiology has only recently been recognized as a field of scientific endeavor; therefore, there are numerous gaps in understanding the ecology of both indigenous and allochthonous microbial populations, as well as the environmental factors governing their survival and activity. By identifying some of those important gaps, directions for future research and development will become apparent.

4.0 SUBSURFACE AS A MICROBIAL HABITAT

Until recently, groundwater has been considered devoid of microbial life (Simon 1983). Early studies in terrestrial microbiology indicated a rapid decline in microbial population with depth (Waksman 1916), and the dogma that microorganisms were, for all practical purposes, absent from regions below the soil profile persisted until the mid-1970s. Furthermore, because of their inaccessibility and remoteness, subsurface environments were not of concern to most environmental agencies or public officials. However, this attitude changed with the growing awareness that groundwater supplies are increasingly becoming contaminated with toxic chemicals and pathogenic microorganisms (Gerba and Bitton 1984).

As contamination of aquifers by organic compounds became an issue of concern, it was recognized by some environmental scientists that the terrestrial subsurface may be inhabited by microorganisms and that these microbial populations may be important in determining the fate of pollutants in groundwater (McNabb and Dunlap 1975; Freeze and Cherry 1979). A comprehensive literature search conducted by McNabb and Dunlap (1975) found numerous reports of microbial activity associated with petroleum deposits and other subsurface formations (Davis 1967; Kuznetsov, Ivanov, and Lyalikova 1963), although little information was available about microbial populations indigenous to water supply aquifers. Since that time, studies of microbiological processes occurring in subsurface environments have been initiated by the Environmental Protection Agency (EPA), the Department of Energy (DOE), the U. S. Department of Agriculture (USDA), and the U. S. Geological Survey (USGS). These studies demonstrate, beyond doubt, that many subsurface environments support a range of microorganisms and that microbial activities are as diverse as those found in surface environments (Fredrickson and Hicks 1987; Ghiorse and Balkwill 1984; Balkwill and Ghiorse 1985; Wilson et al. 1983; Hirsch and Rades-Rohkohl 1984; White et al. 1984; Harvey, Smith, and George 1984; Ehrlich et al. 1984).

To assess the influence that technologies such as ATES have on indigenous microbial populations or on pathogenic organisms that enter groundwater, it

is first necessary to review some of the physical and chemical properties of subsurface environments that may determine the types, abundance, and activities of microorganisms inhabiting these environments.

4.1 ENVIRONMENTAL FACTORS GOVERNING THE PRESENCE AND ACTIVITIES OF MICROORGANISMS IN THE SUBSURFACE

The growth and activity of microorganisms indigenous to the subsurface is governed by a variety of environmental factors. In addition, the nature of the subsurface also has a major role in determining the survival and fate of pathogenic microorganisms and viruses that may be transported to aquifers following the land disposal of sewage, failure of septic tanks and cesspools, stormwater recharge, or agricultural and energy-related activities. The environmental factors that may influence the presence and activities of microorganisms in the groundwater have been reviewed by McNabb and Dunlap (1975) and Bouwer (1984). The factors considered to be the most important are:

- porosity
- adsorption of microorganisms to subsurface particles
- nutrient availability
- redox conditions
- pH
- temperature.

Other factors that may be important include osmotic and hydrostatic pressure, predation, and parasitism.

4.1.1 Porosity

Spatial limitations for microbial presence in the subsurface is determined by the porosity of the various materials present. The size of the individual interstices is the controlling factor and varies widely in subsurface sediments. McNabb and Dunlap (1975) concluded that, with the possible exception of massive crystalline rocks containing no joints or fractures, most subsurface materials provide adequate space to permit the presence of some microorganisms, provided there is sufficient space within the interstices to accommodate the dimensions of the microorganisms.

Spatial limitations are also of concern in the transport of microorganisms into and within the subsurface. This is of particular importance in regard to the transport of pathogenic organisms. The diameter of most bacteria range between 0.5 and 1.0 μm and that of viruses is between 0.03 and 0.3 μm (Bouwer 1984). While adsorption of most viruses and small bacteria is responsible for limiting their movement in the subsurface, the physical straining or filtration of larger bacteria is probably the major limitation to their travel through the subsurface (Bouwer 1984; Gerba and Bitton 1984).

4.1.2 Adsorption

The nature of most subsurface environments (e.g., high specific surface area, low substrate concentration) suggests that bacteria are predominantly attached to solid surfaces (McCarty, Rittmann, and Bouwer 1984; Ghiorse and Balkwill 1984; Hirsch and Rades-Rohkohl 1984; Harvey, Smith, and George 1984). In fact, Harvey, Smith, and George (1984) found that approximately 98% of the subsurface bacteria observed were attached to solid surfaces and that most of these were associated with the fine silt fraction (<20 μm).

McCarty and coworkers (1984) suggest that the advantage provided to attached bacteria is that they will remain in one place and obtain nutrients from the groundwater that flows by. Poindexter (1981) also suggests that the attachment of bacteria to surfaces provides a metabolic advantage in nutrient-poor (i.e., oligotrophic) environments. The association of microorganisms with surfaces has been suggested to elicit a variety of beneficial effects including increased availability of water and nutrients; buffering from environmental stresses such as changes in pH and temperature; and protection from effects of toxic substances, parasitism, and predation (Stotzky 1986).

In addition to the effects on indigenous microorganisms, adsorption is also a factor in the removal of allochthonous organisms, such as small pathogenic bacteria and viruses (Gerba and Bitton 1984). Bacteria and viruses are usually negatively charged at neutral pH, so anion adsorption would appear to be the major mechanism; however, at lower pH, cation adsorption mechanisms may become dominant (Bouwer 1984). In addition, hydrophobic interactions between the cell surface and subsurface sediments may be important (Stotzky 1986).

4.1.3 Nutrient Availability

For microorganisms to survive and function in subsurface environments, an adequate supply of organic and inorganic nutrients must be available for metabolic activity and synthesis of cellular material. Most pristine subsurface environments are characteristically low in dissolved organic carbon (<10 mg L^{-1}) and inorganic nutrients (Girvin et al. 1984; Thurman 1985), providing an oligotrophic environment in which indigenous microorganisms must evolve unique physical and metabolic characteristics to survive and function. Poindexter (1981) summarized some of the properties of oligotrophic bacteria that would contribute to their ability to exploit a low-nutrient environment. These characteristics include small size, which would provide a large surface area to volume ratio, facilitating an increased uptake capacity; a high affinity and low specificity for uptake of a variety of usable substrates; and a versatile dissimilatory capacity (Poindexter, 1981; Ishida and Kadota 1981; Matin and Veldkamp 1978). These characteristics allow indigenous microorganisms to exploit a variety of carbon compounds at low concentrations.

The two principal sources of carbon for subsurface microorganisms include inorganic compounds, principally carbonates, and organic matter incorporated into sedimentary deposits at the time of their formation (McNabb and Dunlap 1975). Other sources include humic, phenolic, naphthalenic, and fatty acids present near petroleum deposits or transported from the surface by recharge water. In addition, organic contaminants entering the subsurface may serve as sources of carbon and energy for indigenous microbial populations (Davis 1967; McNabb and Dunlap 1975; Wilson et al. 1983).

While the quantity of organic compounds in the subsurface is likely to be sufficient to support indigenous microbial activity, it has been speculated that the quality of subsurface organic matter may limit growth (McNabb and Dunlap 1975). The identity of organic compounds in the subsurface is still a matter of speculation; however, it has been suspected that much of the material may consist of humic compounds that are degraded very slowly, or compounds that are not used by microorganisms under conditions found in the subsurface. However, research done to date suggests that this may not be the case. For example, the ability of subsurface microorganisms to utilize a variety of

organic compounds, including natural humic compounds, as well as organic pollutants, has been demonstrated (Fredrickson and Hicks 1987; Wilson et al. 1983; Ladd et al. 1982). Therefore, it appears that indigenous populations have the metabolic versatility to survive and function in the subsurface. Additional research is needed to identify the organic compounds present in groundwaters and the ability of indigenous and allochthonous microorganisms to use those compounds.

Inorganic nutrients, such as nitrogen, phosphorus, sulfur, and trace minerals, are essential for microbial growth. While these elements are probably present in most subsurface environments, their availability may be limited in specific locations where their solubility is low or where adsorption has rendered them unavailable for microbial utilization.

4.1.4 Redox Conditions

One of the most important factors affecting the types and activities of microorganisms in the subsurface is the redox potential of the environment. In oxidized environments, molecular oxygen is used as both the terminal electron acceptor and as a substrate for oxygenase enzymes. As the environment becomes reduced (i.e., anaerobic), due to the utilization of oxygen by heterotrophic bacteria, other electron acceptors are used, including nitrate, ferric iron, oxidized manganese, sulfate, and carbon dioxide. The specific redox potential will influence the type of organisms that can function, the metabolic pathways used, as well as the geochemistry of the various elements in the environment.

The redox conditions of subsurface environments reflect the balance between the rates of microbial metabolism, which result in the reduction of oxygen and other elements, and the rate of entry into groundwater of recharge waters containing those substances. The results of previous research suggest that most pristine subsurface environments contain enough oxygen to support a minimum level of microbial activity (Fredrickson and Hicks 1987; Balkwill and Ghiorse 1985; Wilson et al. 1983). However, in groundwater contaminated with high levels of organic carbon, the available oxygen may be depleted at a rate faster than it can be replenished. In addition, high levels of organic compounds may also deplete all suitable electron acceptors. In this case, the types of

organisms that can survive will be restricted to those with fermentative modes of metabolism.

4.1.5 pH

Variations in groundwater pH will be governed to a large extent by the pH of the recharge water, the activities of indigenous microbial populations, the redox conditions in the subsurface, and the geological formations found within a particular environment (Freeze and Cherry 1979; McNabb and Dunlap 1975). Because of the buffering capacity of HCO_3^- and of minerals such as montmorillonitic clays, extremes in pH are rare in most soils and subsurface sediments (Stotzky 1986). The pH of a particular environment can limit the diversity of microorganisms. Bacteria are most active in near neutral environments, and actinomycetes prefer alkaline environments, while fungi are more active at lower pH levels (Alexander 1977). In addition, the pH of an environment can influence the geochemistry. For example, most heavy metals are more soluble at lower pH levels; therefore, as the pH decreases in a particular environment, the concentration of these metals may rise to levels toxic to microbial growth. Also, the adsorption of toxins, organic compounds, and the microorganisms themselves can be affected by the pH of the environment (Gerba and Bitton 1984).

It should be noted that the pH (as well as the redox) of microenvironments in the subsurface may be completely different from the pH of the bulk solution; thus pH fluctuations near surfaces may greatly affect the activities of subsurface microorganisms even though the bulk pH does not change.

4.1.6 Temperature

The metabolic activities of microorganisms in the subsurface is also controlled by the temperature of that environment. Considerable information is available concerning the relationship between microbial growth and temperature. Mesophilic organisms, which comprise the greatest numbers of microorganisms, have an optimum temperature range of 25 to 40°C. Thermophilic organisms have an optimum temperature between 55 and 60°C, but some can continue to grow at temperatures as high as 75 to 90°C. The maximum temperature that most bacteria (with the exception of heat-resistant spore-formers) can tolerate without being killed is about 100°C, but most microorganisms are killed at

temperatures above 50°C (Atlas and Bartha 1987). The minimum temperature that can support microbial growth appears to be -15°C (Atlas and Bartha 1987). Many psychrophilic microorganisms grow between 0 and 5°C, although the optimum range for many psychrophiles is often 25 to 45°C (Atlas and Bartha 1987).

The normal groundwater temperature range for most drinking-water aquifers corresponds to the mean annual air temperature of the region and ranges between 3 and 25°C (McNabb and Dunlap 1975). The temperature of any particular pristine subsurface environment is not likely to fluctuate over a wide range; therefore, most groundwater environments favor the growth of mesophilic microorganisms.

4.1.7 Other Factors

Groundwaters vary greatly in their osmotic potential [i.e., total dissolved solids (TDS)] and reflect the chemical composition of the formation through which they move. In those regions where the groundwaters have high concentrations of TDS, the growth of microorganisms may be restricted due to decreased water activity and, thus, its availability. However, bacteria differ in their ability to withstand high osmotic pressures, and many organisms are known to tolerate or even require conditions as much as 30% salt (Atlas and Bartha 1987).

Hydrostatic pressure has been suggested as a limiting factor in the growth of microorganisms in the subsurface. Hydrostatic pressure increases with depth at a rate of 1 atm per 10 m; therefore, the hydrostatic pressure of most water-bearing aquifers is likely to remain below 100 atm. Because microorganisms have been shown to tolerate 200 to 300 atm, it is unlikely the hydrostatic pressure will have any great influence on the growth and activities of subsurface microorganisms.

Information on the influence of predators and parasites on subsurface microorganisms, particularly bacteria, is essentially nonexistent. Most earlier studies suggested that subsurface environments are nearly devoid of protozoa and fungi (Ghiorse and Balkwill 1984; White et al. 1984). However, recent studies have demonstrated the existence of protozoa in aquifers (Sinclair and Ghiorse 1987). Significant increases in the numbers of predators or parasites entering the subsurface as a result of transport in recharge waters could

have a pronounced effect on the ecology of these environments. The main effect would be the favoring of those organisms with a high rate of metabolism, as those organisms would be better suited to survive predatory pressure than their slower-growing counterparts.

4.2 MICROBIAL ECOLOGY OF GROUNDWATER

During the past 10 to 15 years, the number of papers that have been published concerning the characterization of microorganisms indigenous to subsurface environments has increased significantly. Before 1975, most of the work published was related to petroleum microbiology, or it was focused on special problems such as iron-depositing bacteria (McNabb and Dunlap 1975). Efforts to study the abundance, diversity, and activities of microorganisms indigenous to domestic-water supply aquifers have been made only recently. Despite this short period of study, a large body of knowledge has been developed concerning subsurface microbiology.

4.2.1 Population Densities

A variety of methods have been used to quantify the numbers of microorganisms present in subsurface environments, including

- direct light and electron microscopy (Ghiorse and Balkwill 1984; Balkwill and Ghiorse 1985; Wilson et al. 1983)
- cultural procedures, such as plate counts, most probable number counts, and enrichment culture techniques (Hirsch and Rades-Rohkohl 1984)
- the use of biochemical indicators [e.g., adenine tri-phosphate (ATP), guanine tri-phosphate (GTP), phospholipids, muramic acids] (White et al. 1984; Smith et al. 1986)
- radioisotope methods (Karl 1986).

Most of these techniques have been adopted from methods commonly used in soil and aquatic microbiological studies; however, it has been necessary to extend the sensitivity of many of these methods due to the low population densities and activities often observed in subsurface samples. For example,

concentration techniques (e.g., filtration) or staining dyes are commonly employed in the direct microscopic examination of groundwater microbial populations (Balkwill and Ghiorse 1985).

Significant numbers of microorganisms, primarily bacteria (but also protozoa and fungi) have been detected in the subsurface. The population densities generally fall in the range of 10^2 and 10^7 cells per g of sediment or per mL of groundwater, with 10^5 to 10^7 being the most common (White et al. 1984; Ghiorse and Balkwill 1984; Balkwill and Ghiorse 1985; Smith et al. 1986; Hirsch and Rades-Rohkohl 1984; Beeman and Suflita 1987; Willis et al. 1975; Webster et al. 1985; Ehrlich et al. 1984; Wilson et al. 1983). The highest numbers of microorganisms are usually detected in sandy aquifer sediments, while most unsaturated zones and clay aquifer sediments harbor low populations of microorganisms.

The total cell counts of subsurface sediments remain remarkably constant with depth (Balkwill and Ghiorse 1985). However, the viability of microorganisms detected in the subsurface is considerably more variable. A tetrazolium dye reduction variation of the direct counting procedure has been used to estimate the proportion of active microorganisms in subsurface environments (Webster et al. 1985). Using this technique, viable cell counts have been shown to range from essentially zero to values as high as the total microscopic counts (Webster et al. 1985). This variability in viable cell counts may reflect the trophic status of a particular subsurface environment.

It should be noted that most of the research on the microbiology of subsurface environments has been concerned with shallow aquifers, and relatively few reports have been made on the microbiological status of deep subsurface environments (>50 m). However, recent evidence has demonstrated that, at least in some deep subsurface environments, the population density, diversity, and activity can equal or surpass that of shallow aquifer environments (Fredrickson and Hicks 1987).

4.2.2 Physiological Groups

Bacteria present in subsurface environments have been shown to represent a range of diverse physiological groups. In addition, protozoa and fungi

have also been reported (Hirsch and Rades-Rohkohl 1984; Sinclair and Ghiorse 1987). Some of the different physiological groups that have been detected in subsurface environments include chemoheterotrophs, nitrifying bacteria, hydrogen-oxidizers, nitrogen-fixers, sulfur-oxidizers, iron-oxidizers, and reducers (Fredrickson and Hicks 1987; Hirsch and Rades-Rohkohl 1984; Wilson et al. 1983; Ehrlich et al. 1984). Both aerobic and anaerobic microorganisms have been observed in subsurface environments (Fredrickson and Hicks 1987; Balkwill and Ghiorse 1985; Wilson et al. 1983; Ghiorse and Balkwill 1984; White et al. 1984; Willis et al. 1975).

The physiological diversity of the subsurface suggests that a unique microbial ecosystem has evolved within these environments. Little is known about how perturbations, such as the introduction of contaminants or ATES waters, will affect this ecosystem. While the total population density of contaminated subsurface environments has been shown to be similar to pristine environments (Ehrlich et al. 1984), shifts in population diversity are likely to occur.

4.2.3 Metabolic Activity

Metabolic studies using subsurface sediment samples and pure cultures of subsurface microorganisms, or consortia, have shown that the subsurface harbors a unique microbial population with broad metabolic capabilities. The potential of subsurface populations to utilize both natural and anthropogenic organic compounds under aerobic, denitrifying, sulfate-reducing, and methanogenic conditions has been demonstrated (Wilson et al. 1983; Kuhn et al. 1985; Fredrickson and Hicks 1987; Suflita and Miller 1985; Lee, Wilson, and Ward 1984; Gibson and Suflita 1986; Rittmann, McCarty, and Roberts 1980; Zoeteman, DeGreef, and Brinkmann 1981; Bennett et al. 1985). The metabolic activity of subsurface microbiota from pristine environments may be low compared to that of contaminated environments (Herbes and Schwall 1978), reflecting the carbon-limiting status of most pristine subsurface environments.

The genetic information for the metabolism of a variety of anthropogenic compounds are carried on bacterial plasmids (Chatterjee and Chakrabarty 1981). Evidence has shown that subsurface microorganisms harbor plasmids. In addition,

recent evidence has suggested that these bacterial plasmids may be involved in antibiotic and metal resistance of subsurface microorganisms, as well as the degradation of quinoline (Hicks et al. 1987).

4.2.4 Pathogenic Organisms

Between 1946 and 1977, there were approximately 62,273 reported cases of waterborne diseases attributed to contaminated groundwater in the U.S. (Keswick 1984). Groundwater can become contaminated when it comes in contact with fecal material or sewage containing pathogenic bacteria or viruses. Although normally associated with a host, many pathogenic bacteria are capable of surviving outside their hosts. There is concern that ATES systems may provide a route for the release and propagation of pathogenic organisms in subsurface environments; therefore, a brief discussion of the literature describing pathogenic organisms found in these environments is warranted.

The survival, growth, and transport of bacterial and viral pathogens in subsurface environments has been the topic of numerous review articles (Gerba and Bitton 1984; Gerba 1985; Matthess and Pekdeger 1985; Keswick 1984). The presence of pathogenic bacteria in environmental samples is usually assessed by measuring the number of coliforms, especially fecal coliforms, present in the sample. Extensive bacteriological surveys on groundwater quality in the U.S. indicate that 9 to 85% of the samples examined contained coliform, and 2 to 75% of them contained fecal coliforms (Gerba 1985). Some of the specific bacterial pathogens that have been identified include Salmonella, shigella, Vibrio chlorerae, and Escherichia *coli* (Keswick 1984).

More than 100 different types of viruses have been found in sewage, including poliovirus, hepatitis A, echo, coxsackie, rota, adeno, and Norwalk-like viruses (Keswick 1984). Until recently, adequate methods for the detection and identification of enteric viruses in groundwater were not available. However, many human pathogenic viruses most certainly exist in groundwater, based on the large number of disease outbreaks associated with these viruses and attributed to contaminated groundwater (Gerba 1985). New techniques have allowed the isolation of most of the above viruses from groundwater (Wellings, Mountain, and Lewis 1977; Hejkal et al. 1982; Gerba 1985).

The primary sources of pathogenic contaminants in groundwater are sewer leakage, animal wastes from feedlots, septic tanks and cesspools (Keswick 1984). Other potential sources include solid wastes from municipal operations, polluted precipitation, and industrial cooling tower waters. The role of ATES systems in transporting pathogenic organisms to and from groundwater has not been investigated. Chill ATES systems that operate on an open cycle mode would seem to have the greatest potential for transporting pathogenic microorganisms, as the direct contact of groundwater with air blown through cooling towers during operation increases the potential for dissemination. Cooling towers are a known source of many different microorganisms including a wide variety of bacteria, fungi, and algae (McCoy 1980). In addition, in at least three recorded outbreaks of Legionnaires' Disease (caused by bacteria of the genus Legionella), the waters in cooling towers or evaporative condensers of air conditioning systems were implicated as the source of the infection.

Numerous environmental factors affect the survival of pathogenic bacteria and viruses in subsurface soil and are listed in Tables 1 and 2.

TABLE 1. Factors Affecting the Survival of Pathogenic Bacteria in the Subsurface[a]

Factor	Comment
Moisture content	Greater survival in moist soils
Temperature	Longer survival at low temperatures
pH	Shorter survival time in acid soils
Organic matter	Increased survival with higher organic matter content
Antagonism from other biota	Increased survival time under sterile conditions

(a) Adapted from Gerba and Bitton (1984).

TABLE 2. Factors Affecting the Survival of Viruses in the Subsurface[a]

Factor	Comment
Temperature	Longer survival at low temperatures
Moisture	Less survival at lower moisture
pH	Shorter survival with increasing pH
Adsorption	Increased survival with greater adsorption
Cations	Survival increases with higher levels of certain cations (i.e., Al)
Antagonism from other biota	Aerobic organisms adversely affect virus survival, while anaerobes have no effect

(a) Adapted from Gerba and Bitton (1984) and Gerba (1985).

The two principal factors influencing the survival of pathogenic bacteria and viruses in groundwater are temperature and water content. The survival of microorganisms is greatest at low temperatures; below 4°C they can survive for months or even years (Gerba 1985). The mortality rate for both bacteria and viruses is approximately doubled with each 10°C increase between 5 and 30°C (Reddy, Khaleel, and Overcash 1981). Previous reports show that above 30°C, virus survival is limited; however, viruses adsorbed to sediment particles are usually protected (Gerba 1985).

Survival of both bacteria and viruses is greater under saturated conditions, and there is marked inactivation during drying periods. Therefore, saturated conditions (i.e., aquifers) favor the survival of these organisms.

5.0 IMPACT OF ATES SYSTEMS ON GROUNDWATER MICROBIOLOGY

Previous environmental assessments (EA) of ATES systems have determined that there were no significant adverse environmental impacts caused by the implementation of this technology (Reil ly 1980). However, the impact of ATES on indigenous and allochthonous subsurface microorganisms was not determined in the original EA. Aquifer thermal energy storage systems have the potential to affect indigenous microbial populations, either adversely or beneficially, by altering the chemical and/or physical environment (i .e., changing the temperature, altering the chemical composition of the environment, or altering the interactions of microorganisms with subsurface minerals). In addition, ATES systems may have the potential for propagation and release of pathogenic organisms, including *Legionella*.

This section attempts to identify some of the potential effects, both adverse and beneficial, of ATES systems on microbiota in aquifers. It should be recognized that, at present, no research has been done to determine the effects of ATES technology on microorganisms. Furthermore, the microbial ecology of groundwater environments is poorly understood. Therefore, the assessment presented in this section is speculative in nature and represents a combination of our understanding of environmental factors that affect microbial behavior in general and the effect of ATES systems on those factors. Sections 5.1 and 5.2 address the effects of ATES technology on indigenous and allochthonous microbiota. Section 5.3 addresses the effects of ATES on the propagation and release of *Legionella*. *Legionella* is emphasized because of its pathogenicity and because it is ubiquitous in aquatic environments.

5.1 POTENTIAL EFFECTS ON INDIGENOUS MICROBIAL POPULATIONS

As stated earlier, there are no data showing the effect of ATES systems on microorganisms in groundwater. Therefore, an assessment of the potential adverse or beneficial effects must be based on the known or perceived changes in the physicochemical properties of groundwater environments caused by the implementation of ATES systems and the present understanding of how those physicochemical properties influence the growth and activities of microorganisms.

5.1.1 Temperature

The greatest change in groundwater properties caused by the use of ATES systems will occur in the temperature of the aquifer. Aquifer thermal energy storage systems fall in two basic categories: 1) heat systems whereby heated water (up to 250°C) is injected into the aquifer during the summer and recovered during the winter as a heating source, and 2) chill systems that inject and recover cold water. The greatest effect on indigenous microbial populations is likely to be caused by the high-temperature ATES systems. In systems like the St. Paul field test facility (Raymond and Kannberg 1986), the mean injected water temperature is 108.5°C. Most microorganisms are killed rapidly at temperatures above 75°C. The approximate thermal death times range from 20 to 30 minutes at 57°C for sensitive microorganisms to 100 to 330 minutes at 100°C for resistant spore-formers (Atlas and Bartha 1987). Only a few bacterial species are able to survive and grow at temperatures above 75°C, and these organisms are usually associated with hot springs or hot deep-sea vents, not with groundwaters (Atlas and Bartha 1987). Therefore, the obvious effect of high-temperature systems on indigenous microbial populations would be the reduction in numbers of microorganisms in the aquifer. In addition, if organisms survive, the species diversity will probably be reduced greatly.

The ramifications of reduced populations and species diversity are unknown. However, increasing evidence suggests that these organisms may be important in the degradation of environmental contaminants entering groundwaters (Fredrickson and Hicks 1987; Hicks et al. 1987; Wilson et al. 1983). Reduction of indigenous populations caused by the widespread implementation of high-temperature ATES systems may increase the environmental damage caused by the release and movement of these pollutants. Further research is needed to quantify the extent of indigenous microbial population reduction likely to be caused by implementation of ATES systems and the effects this reduction will have on contaminant behavior.

While thermal ATES systems are likely to lead to a reduction in microbial activity, the extent of this reduction is unknown. Thermally affected zones are generally small (confined to a zone typically less than 100 m in diameter

around the injection wells), and it is unknown if changes occurring in a small zone within the aquifer will greatly affect the overall biogeochemical processes of the aquifer.

Chill systems, such as the one in operation in Tuscaloosa, Alabama, operate between 2 and 16°C (Raymond and Kannberg 1986). The effects of these temperatures are not expected to be the same as with high-temperature systems. Although most aquifer microorganisms appear to be mesophiles, many are psychrophilic or psychrotrophic and can survive and grow at low temperatures. Even if the temperature prohibits growth, most indigenous microorganisms are likely to survive the reduced temperatures. The major effect of the chill system will probably be a reduction in the activity of the indigenous microbial populations. As with the high-temperature systems, this reduced activity may decrease the capability of aquifers to remove environmental contaminants. On the other hand, the concentration of O_2 in the aquifer may increase due to increased O_2 solubility; thus increasing the aerobic activities of indigenous microbial populations and their ability to degrade pollutants. It is not known which effect (i.e., reduced activity because of low temperature or increased activity because of higher O_2 concentrations) will be dominant.

5.1.2 Changes in Groundwater Chemistry and Physical Properties

Changes in water chemistry caused by the implementation of ATES systems have the potential to influence the activities and populations of indigenous microorganisms. Most minerals are more soluble at higher temperatures; therefore, an elevation in concentration of the normal ion suite of the groundwater is likely to occur. Notable exceptions include $CaCO_3$, $Ca_3(PO_4)_2$, $CaSO_4$, and $FePO_4$ (Snoeyink and Jenkins 1980). It is not known if the increase in ionic strength will be large enough to have a significant adverse or beneficial effect on indigenous microorganisms. Furthermore, it is unlikely that changes in groundwater chemistry would dominate the direct effect of temperature changes on the microbial population.

The situation in cold-water systems may be different. As mentioned previously, the O_2 concentration in these systems may be elevated. The increased oxidation status of the environment would change the chemical species available to microorganisms, as well as changing the types of activities performed by the

microbial populations. It is unknown if these changes will cause beneficial or adverse changes to the environment or to microbial populations within the environment.

Another potential change to groundwater chemistry caused by the implementation of ATES systems is the elevated concentration of organic and inorganic compounds caused by their introduction during operation (Reilly 1980). The chemical composition of injection waters is not known, hence the effect on subsurface microorganisms is not known. If elevated levels of organic compounds are associated with these waters (i.e., from the chemical treatment of circulating waters), indigenous microbial activity may be stimulated, thus increasing the potential of these populations to reduce the organic levels in the aquifer. On the other hand, the introduction of organic compounds may decrease activity for two reasons; first, the introduced compounds may be toxic to the indigenous population, and second, most groundwater microorganisms appear to be oligotrophic, and increased organic carbon content may decrease their activity by shocking their systems due to the increased substrate supply (Poindexter 1981; Balkwill and Ghiorse 1985).

Changes in the pH of the aquifer that can occur following the implementation of ATES technology are also unknown. However, changes that cause the pH to decrease or increase beyond the range suitable for microbial growth (i.e., pH levels between 5.5 and 8.0) are likely to have adverse effects. In addition, changes in pH may cause major changes in the genera of microorganisms that dominate the aquifer.

Other changes that may affect microbial activity include changes in the permeability of the aquifer and changes in the adsorptive capacity of the aquifer. Changes in the permeability of the aquifer will alter the flux of nutrients to microorganisms, as well as their mobility. Whether these changes would be beneficial or detrimental is unknown. Most subsurface organisms are attached to surfaces (Harvey, Smith, and George 1984), which appears to increase their ability to survive and grow under low nutrient conditions (Poindexter 1981). Therefore, changing the adsorptive capacity of an aquifer may be detrimental to indigenous microorganisms if it decreases their ability to attach to aquifer surfaces.

5.2 POTENTIAL FOR PROPAGATION AND RELEASE OF PATHOGENS

In addition to the concern over possible deleterious effects to indigenous microbial populations caused by the implementation of ATES systems, there is also concern that this technology may encourage the propagation and release of pathogenic microorganisms. This concern arises because pathogenic organisms are often isolated from groundwaters as well as from structures used in some ATES systems (e.g., cooling towers), and the environmental conditions created by ATES systems, particularly chill systems, may favor the survival of pathogenic microorganisms (McCoy 1980; Keswick 1984; Gerba 1985). Some of the pathogenic organisms isolated from groundwater and cooling towers include Salmonella typhi, Shigella dysenteriae, Vibrio cholerae, *Legionella* pneumophila, Giardia *lamblia*, as well as a variety of viruses (McCoy 1980; Keswick 1984; Gerba 1985). Although most pathogenic microorganisms are not indigenous to the subsurface, they often survive for long periods of time within groundwaters and water cooling systems. In view of the large number of reported disease outbreaks that have been traced to water systems contaminated with pathogenic microorganisms (see Section 4.2.4), an environmental assessment of the impact of ATES on pathogenic organisms is warranted. An assessment of the potential influence of ATES technology on pathogenic organisms, in general, is made in this section. Section 5.3 discusses specifically the potential for dissemination and survival of Legionella in the subsurface as a result of ATES technology implementation. Attention has been focused on *Legionella* because it is ubiquitous to aquatic environments and because of its pathogenicity. In addition, recent evidence has linked the outbreak of Legionnaires' Disease with contaminated groundwater or cooling-tower water.

Tables 1 and 2 describe some of the environmental factors affecting the survival of pathogenic microorganisms in groundwater. Temperature is probably the most important environmental factor governing the survival of pathogens (Keswick 1984). As stated earlier, hot-water ATES systems (>100°C) are not conducive to the survival and growth of microorganisms; therefore, it is also unlikely that pathogenic microorganisms would survive those environments. However, chill systems (such as the one in operation in Tuscaloosa, Alabama)

may be conducive to pathogen survival. Both pathogenic bacteria and viruses survive best at temperatures around 3 to 10°C, which is also the temperature range for the chill ATES system in Alabama. Therefore, the operation of such systems would appear to favor the survival of most pathogens if they are present in the groundwater or introduced with the injection water. In addition, those factors (e.g., moisture, oxygen, pH) that influence the survival of pathogens do not appear to limit the survival of pathogens in chill ATES systems. Furthermore, chill ATES systems tend to suppress the activities of indigenous microorganisms (see Section 5.1.1), which may reduce antagonistic interactions with pathogens.

While the survival of pathogenic microorganisms may be enhanced in chill ATES systems, it is unknown if the use of ATES systems will be responsible for their release into the subsurface. Two of the more important factors governing movement and colonization of microorganisms in the subsurface are adsorption and flow rates (Keswick 1984). Microbial movement increases with decreasing adsorption and increasing flow rates. The effect of temperature on the adsorption of bacteria and viruses to surfaces is unknown. A variety of mechanisms are involved in the attachment of bacteria and viruses to surfaces, including ion exchange, flocculation, and physical and chemical adsorption; therefore, it is reasonable to assume that temperature has some role in determining the adsorption of bacteria.

The flow rate of groundwater will probably increase following implementation of ATES systems. Therefore, some potential exists for the migration of pathogens. Additional research in the factors governing movement and colonization of microbes in the subsurface is needed before an accurate assessment can be made of the effect of ATES systems on the release of pathogenic microorganisms.

5.3 POTENTIAL FOR PROPAGATION AND RELEASE OF LEGIONELLA SPP.

5.3.1 Prevalence of *Legionella* in Aquatic Environments

Legionella pneumophila, the causative agent of Legionnaires' Disease, has been reported to be prevalent in both man-made and natural aquatic environments (Fliermans et al. 1979, 1981; Fliermans 1984; Morris et al. 1979; Tison

et al. 1980). Numerous studies have indicated a strong association of the incidence of legionellosis with cooling systems (e.g., cooling towers, exchangers, and wash systems) (Orrison, Cherry, and Milan 1981; Cordes et al. 1980; Dondero, Rendtorff, and Mallimi 1980; Miller 1979) and potable water systems (Reasoner 1983; Stanwick 1986). In a 1978 outbreak of Legionnaires' Disease in Memphis, studies conducted by Dondero, Rendtorff, and Mallimi (1980) indicated that the auxiliary cooling tower, which had not been used for 2 years and had not been chemically treated, harbored *Legionella* spp., while the shut-down tower, which had been used prior to the disease outbreak, was free of the bacterium. Tracer smoke analysis indicated that the *Legionella* bacterium was transmitted through the air. In addition, it is well documented that association of outbreaks of legionellosis with cooling towers has proven to be negative when *Legionella* could not be cultured (Broome and Fraser 1979; Fisher-Hock et al. 1981).

Legionella has been isolated from different types of cooling towers from a variety of locations. Tyndall (1982) conducted a survey of both industrial and air conditioning cooling towers for the presence of bacteria. Four different types of cooling towers at various locations in the continental U.S. were examined. The types of towers sampled included: 1) large, continuous mechanical and natural draft; 2) large, intermittent; 3) continuous air conditioning; and 4) small intermittent air conditioning systems. The source water was sampled and characterized for *L. pneumophila.* Data collected showed that *Legionella* spp. disease bacterium (LDB) were found in the majority of cooling waters at concentrations of 10^4 bacteria per liter of water. River water used as source water had more than 10^5 LDB per liter, while tapwater had less than 10^4 LDB per liter. Some of the cooling towers tested over a period of years maintained 10^5 to 10^7 LDB per liter of water.

Fliermans et al. (1981) also identified *Legionella* spp. at extremely low concentrations (approximately 1% of the total bacterial population) from natural habitats not associated with the outbreak of the disease. In this study, 67 different U.S. rivers and lakes (a total of 793 samples) were sampled and evaluated by direct fluorescent antibody (DFA) technique. All of the samples examined were found to contain *L. pneumophila.*

Because of the association of cooling towers and air conditioning systems with the outbreaks of Legionnaires' Disease, as well as its widespread distribution in nature, the potential for association of ***Legionella*** with ATES systems should be considered. Understanding the epidemiology and the ecology of legionellosis and ***Legionella*** bacteria will assist in this evaluation.

5.3.2 Epidemiology

Two distinct clinical presentations of the disease caused by ***L. pneumophila*** have been recognized: Legionnaires' Disease and Pontiac Fever (Broome and Fraser 1979; Kaufmann et al. 1981). Legionnaires' Disease is a multisystem illness typically including pneumonia and is potentially fatal. Pontiac Fever has been described as a nonpneumonic, self-limiting illness with myalgia.

Other distinguishing differences between the two forms of legionellosis are the incubation period and the attack rate. Legionnaires' Disease usually begins 2 to 10 days after exposure. Symptoms include high fever, muscle aches, chest and abdominal pain, and shortness of breath. In 90% of the cases, pneumonia is present, and approximately 20% of the cases are fatal., either due to progressive pneumonia or septic shock (Fraser and McDade 1979). Most patients require hospitalization and antibiotic therapy.

Pontiac Fever has clinical symptoms similar to those of Legionnaires' Disease, but patients are ill from 2 to 5 days with an average incubation period of 36 hours, generally do not require antibiotic therapy, and usually recover from the disease (Muder, Yu, and Woo 1986). To date, no microbiological characteristics or epidemiological settings have been identified that can explain the difference in the clinical presentations or the epidemiological findings. Possible explanations of these differences might be in the virulence factor specific to the organisms involved, or that Pontiac Fever may be a response to nonviable cells (Fraser et al. 1979).

5.3.2.1 Occurrence of Disease

Since legionellosis was recognized as a new disease, entity cases have been discovered wherever they are sought. In a report by Fraser and McDade (1979), evidence of legionellosis was shown in 40 states in the U.S. and also in Australia, Canada, Denmark, Greece, Israel, Italy, The Netherlands, Norway,

Spain, Sweden, Switzerland, West Germany, the United Kingdom, and Yugoslavia. There appears to be no significant geographical pattern to the disease. However, passive surveillance at the Center for Disease Control (CDC) in Atlanta, Georgia, indicates that there is variation in the attack rate, as 10 of the 13 states with the highest attack rates are east of the Mississippi River (England et al. 1982). There is some evidence that a cluster of cases have a common source and that almost all of the cluster and most of the sporadic cases have been reported in the summer. Only one study has examined the proportion of pneumonia cases due to *Legionella* spp. in various studies of the etiology of pneumonia. Foy and coworkers (1979) examined individuals enrolled in the health program at the University of Washington School of Public Health. Their analysis indicated that *L. pneumophila* was the cause of 12 cases per 100,000 population each year. With this incidence rate, on a nationwide basis there could be 26,000 cases of legionellosis in the U.S. each year.

Generally, middle-age and elderly individuals are more susceptible to Legionnaires' Disease, although children as young as 16 months have been diagnosed with the disease (Broome and Fraser 1979). Generally, more men than women are afflicted, and usually cigarette smokers and heavy drinkers are more prone to the disease. Those individuals who have other medical problems such as cancer, leukemia, lymphoma, and kidney failure have a predisposition to this disease, as well as to other types of infection.

Sporadic cases of Legionnaires' Disease are more frequent among travelers, construction workers, and people living near sites of excavation or construction (Storch et al. 1979). It is thought that these individuals are more likely to be exposed to airborne dust that carry the bacteria. Many outbreaks of legionellosis have occurred in or near a hotel or hospital. In some of the outbreaks, these buildings are simply a place where susceptible people gather; in other cases, the central air conditioning system has been implicated as the possible source of the bacteria.

5.3.2.2 Mode of Transmission

Legionella pneumophila may be able to contaminate cooling towers by airborne routes (Fraser 1980). To effectively cool the circulating water, large volumes of air are drawn through the tower, allowing dust and other particulates

to be deposited in the water. It seems reasonable that airborne microorganisms also could be easily introduced into the cooling-tower waters. Because cooling towers are usually open structures, other sources of contamination can occur, including droppings and detritus of insects and birds. It is not clear whether the cooling towers are simply collecting devices, or if they provide the necessary environment for propagation of microbial contaminants. However, it seems reasonable that the powerful aerosol created as a byproduct of the tower operation could pollute the local atmosphere with microbial contaminants and that the cooling tower itself could provide the right ecological environment for the proliferation of microorganisms, including ***Legionella.***

5.3.3 Ecology of *Legionella*

There is considerable evidence that *Legionella* is part of the natural microbial community of aquatic environments and accounts for only 1% of the total population (Fliermans et al. 1979). *Legionella* spp. have been shown to survive in a variety of environments. *Legionella* spp. have been isolated from habitats with a wide range of physicochemical characteristics including:

- temperatures between 5.7 and 63°C
- pH values between 5.5 and 8.1
- dissolved oxygen content between 0.3 and 9.6 ppm (0.3 to 9.6 mg/ml)
- specific conductivities between 18 and 106 µs/cm
- chlorophyll content between 0.7 and 24.0 mg/m^3
- pheophytin content between 0.2 and 18.8 mg/m^3.

It has also been shown that low levels of certain metals (such as iron, zinc, and potassium) enhance the growth of naturally occurring *L. pneumophila* (States et al. 1985; Reeves et al. 1981).

The temperature of aquatic environments certainly influences the activity of microorganisms, as well as their survivability. Fliermans and coworkers (1981) reported that *Legionella* spp. is more readily isolated from naturally warm and thermal habitats than from cooler environments. It has been proposed that *Legionella* spp. are similar to mesophilic and thermophilic organisms in their composition of branched-chain fatty acids (Fliermans 1984). Other studies (Wadowsky et al. 1985) indicate that multiplication of *Legionella* may occur

across a wide range of temperatures (i.e., 25 to 45°C). Wadowsky et al. (1982) isolated *Legionella* spp. from hot water tanks maintained between 30 to 54°C but not from those maintained between 71 and 77°C. Stout et al. (1985) suggested that the *Legionella* spp. has a greater predilection for hot-water systems than for cold-water systems, as evidenced by the number of strains that exhibited satellitism.

Satellitism is defined as nutritional symbiosis between *Legionella* spp. and the other environmental microorganisms (Wadowsky and Yee 1983). It is thought that the environmental organisms supply *Legionella* spp. with essential nutrients (i.e., amino acids) needed for growth. Some of the microflora associated with this phenomenon have been identified as various species of the genera *Flavobacterium*, *Pseudomonas*, *Alcaligenes*, and *Acinetobacter*. However, this relationship is not reciprocal (i.e., improved growth of the other organisms does not occur in the presence of *Legionella* spp.).

In addition to nonspecific satellitism, *Legionella* spp. also has been isolated from aquatic environments with which specific microorganisms are always associated (Pope et al. 1982; Tison et al. 1980; Berendt 1981). *Fisherella* spp., a blue-green alga, is frequently present in water towers and cooling systems associated with outbreaks of Legionnaires' Disease and is thought to enhance the survivability of *L. pneumophila* in an aerosolized form by protecting *Legionella* from desiccation, probably by encapsulation with polysaccharides. Dissemination of *Legionella* from cooling towers may be enhanced by the association with the algal component. In addition, survival of *Legionella* spp. is influenced by relative humidity (Berendt 1980). In studies reported by Tison et al. (1980), *Fisherella* spp. supplied nutrients required for survival of *Legionella* bacteria. Growth rates of *Legionella* spp. in co-culture with the blue-green algae were equal to growth rates observed in a complex medium. Other investigators (Pope et al. 1982) have shown that several other algae and cyanobacteria can support the growth of *L. pneumophila* in media deficient in organic nutrients other than those supplied by the algae. Borach and Snyder (1983) have reported that specific fractions from a cyanobacterial culture supernatant stimulated the oxygen uptake by *L. pneumophila*. Those fractions that stimulated the oxygen uptake contained protein as their major component,

as well as a carbohydrate. This interrelationship of *Legionella* spp. with other environmental microorganisms may provide an explanation for the widespread distribution of *Legionella* spp.

A more recent study (Wadowsky et al. 1985) of the multiplication of *L. pneumophila* in potable water systems indicates that *L. pneumophila* can grow over a wide range of pH values (i.e., 5.5 to 9.2) when in co-culture with other naturally occurring bacteria. However, Feeley et al. (1979) reported that the pH range was narrow (between 6.6 and 7.5) for agar-maintained stock cultures. The data from Wadowsky and coworkers (1985) suggest an association between the multiplication of naturally occurring *L. pneumophila* and other environmental organisms. Extreme levels of pH (i.e., 5.0 or 10.5) have been shown to limit the multiplication of the environmental bacteria and also inhibited the multiplication of *L. pneumophila*. However, *L. pneumophila* multiplied at a pH of 5.5, while the other environmental bacteria failed to multiply. This observation suggests that there are other microorganisms present that were not detected by the culture methods used or that the dead biomass released nutrients that were used by *L. pneumophila*.

It has been reported that *Legionella* spp. are ingested by amoebae and can multiply intracellularly (Anand et al. 1983; Rowbotham 1980). Until recently, studies to determine if these microorganisms are present during an outbreak of legionellosis have not been conducted. Barbaree and coworkers (1986) isolated an amoeba and two ciliates (*Tetrahymena* spp. and *Cyclidium* spp.) from cooling-tower waters containing *L. pneumophila* at the site of a legionellosis outbreak. Both the *Tetrahymena* spp. and the amoeba consistently showed the ability to support intracellular multiplication of *L. pneumophila*. The ability of *L. pneumophila* cells to reside inside free-living protozoa is a possible mechanism for the persistence of these bacteria during adverse conditions. This protective mechanism may explain the inability of some biocides to effectively eliminate *L. pneumophila* from contaminated towers and also may explain the survivability of *Legionella* spp. during the winter.

Another environmental factor that seems to influence the survival and growth of *Legionella* spp. in aquatic systems is the presence of sediment. Stout et al. (1985) showed a direct correlation between the concentration of

sediment and the survival and propagation of *L. pneumophila* in hot-water systems (i.e., as the concentration of sediment was reduced, the survival of *L. pneumophila* also declined). In addition, their data indicated that the combination of sediment and microflora had a greater effect on the survival of *L. pneumophila* than either the sediment or microflora alone. Wadowsky and coworkers (1982) proposed that *L. pneumophila* concentrate and multiply at the bottom of hot water tanks by adhering to the sediment that accumulates there. States et al. (1987) reviewed the potential for *L. pneumophila* to survive and multiply in municipal drinking-water systems and proposed that the bottoms and corners of reservoirs and sedimentation basins could accumulate metabolites of other bacteria and algae to support the growth of *Legionella* spp. Attachment to sediment, either in groundwater or particles in cooling towers, is beneficial to the survival of *Legionella* spp. because these surfaces often accumulate nutrients needed for the bacteria's growth (McCarty, Rittmann, and Bouwer 1984).

Although most data indicate that the interrelationships with other microflora, sediment, pH, and temperature are significant factors in the survival and propagation of *Legionella* spp., *L. pneumophila* has been shown to persist in tap and distilled water and survival is influenced mainly by the dissolved oxygen concentration. Data collected by Skaliy and McEachern (1979) demonstrated that strains of *L. pneumophila* can survive up to 129 days in distilled water and 369 days in tapwater. Temperature was not an experimental variable in these studies. Other studies (Wadowsky et al. 1985) have shown that *L. pneumophila* can multiply in tapwater at 35°C but only under aerobic conditions. In these investigations, viable counts of naturally occurring *L. pneumophila* incubated in tapwater saturated with oxygen and in untreated tapwater increased 1.0 log colony-forming units (CFU)/ml within 7 days. Under anaerobic conditions, viable cell counts decreased over time. These studies and others (Yee and Wadowsky 1982) demonstrate that not only is *L. pneumophila* able to survive in tapwater and distilled water, but it can multiply in sterile water.

5.3.4 Isolation and Detection of *Legionella*

One of the major obstacles to studying the ecology of *Legionella* spp. in either clinical or environmental samples, is that these samples usually

harbor other bacteria and fungi that obscure the detection of *Legionella* isolates. Consequently, a number of different selective media and pretreatment procedures have been developed. A base medium, charcoal yeast extract (CYE) in combination with antibiotics, has been used as a selective medium in a few studies (Feeley et al. 1979). Edelstein (1981) evaluated antibiotics (e.g., cefamandole, polymycin B, and anisimycin) in combination with an organic buffer (ACES buffer) and alpha-ketoglutarate in the CYE medium. This study showed that media that contained all of the above components greatly enhanced the recovery of *L. pneumophila* from clinical samples; however, a less dramatic recovery of *L. pneumophila* from environmental samples was observed. To enhance the recovery yield from environmental samples, samples are acid-treated prior to plating onto the CYE medium. This treatment consists of diluting the sample 1:10 in 0.2 M HCl-KCl buffer with a pH of 2.2. This mixture is then incubated at 21°C for 4 min, followed by plating onto the CYE containing the appropriate antibiotics. For most samples, the recovery yield of *Legionella* spp. is improved due to the reduction of other environmental microorganisms; however, in some samples, acid-washing decreases the yield. In general, environmental samples should be analyzed with and without acid pretreatment.

Other investigators (Bopp et al. 1981), have developed similar procedures (i.e., low pH and selective media) for isolating *Legionello* spp. from environmental samples. All samples tested positive for *Legionella* spp. The selective media used by Bopp et al. (1981) consists of CYE agar to which the following antibiotics were added: cephalothin, colistin, vancomycin and cycloheximide. Acid treatment of the samples is similar to that described above. However, samples are concentrated by centrifugation prior to acid treatment. This concentration step seems to improve the recovery of *Legionella* spp. from environmental samples. Use of selective medium, in combination with the acid buffer treatment, gave a recovery of 45%, while selective medium alone gave a recovery of 36%.

Vickers, Brown, and Garrity (1981) described a similar medium to which bromcresol purple and bromothymol blue dyes were added to differentiate the various members of the family *Legionellaceae*. The medium was designed for the separation of species of *Legionello* from each other rather than for the

purpose of isolating ***Legionella*** spp. from the environment. Although this medium is useful for pure culture studies, confirmational biochemical, serological, and genetic techniques should also be used for environmental and clinical samples.

In early attempts at isolating the etiological agent of Legionnaires' Disease from clinical specimens, egg yolk embryos were used. A wide variety of pathogenic organisms, including ***Legionella*** spp., have been isolated by this method. This method has been used primarily to maintain the virulence of the organism, because passage of ***Legionella*** isolates on bacteriological media reduces its virulence for either guinea pigs or for embryonated (i.e., fertilized) eggs. However, the use of embryonated eggs is not appropriate for isolating or enriching ***Legionella*** spp. from the natural environment, because other microorganisms that are capable of using the nutrients in the embryonated eggs will interfere with ***Legionella*** isolation. Also, the generation time of ***Legionella*** (approximately 100 min in the laboratory) is much slower than the generation time of most naturally occurring aquatic microorganisms.

Direct fluorescent antibody (DFA) techniques are the most frequently used methods for screening water samples for the presence of ***Legionella*** spp. (Fliermans et al. 1981). They are sensitive and accurate for use in aquatic systems but should be used in conjunction with other isolation techniques. From an ecological viewpoint, there is no other technique in which a particular bacterium can be viewed directly and identified, without selectively enriching for the organism from its in-situ habitat. The DFA technique is extremely useful in evaluating the density, serology, and distribution of selected organisms under in-situ conditions. Unfortunately, this technique does not distinguish between living and dead cells. Consequently, a need to establish the number of viable cells in the environment is also required.

Tetrazolium dyes (i.e., 2-p-iodophenyl-3-P-nitro-phenyl tetrazolium chloride, INT) in combination with the DFA, have been used to differentiate between viable and nonviable cells (Fliermans, Soracco, and Pope 1981; Zimmerman, Iturriaga, and Becker-Birch 1978). The oxidized form of the dye is placed in the water sample and is absorbed by actively respiring cells and deposited inside the cells. The oxidized form of the dye acts at the ubiquinone-

cytochrome b complex level in the electron transport system to form the reduced INT compound, formazan. If the electron transport system of the prokaryotic cells is active (i.e., if the cell is viable), crystals of formazan will be deposited in the cell and can be seen under a bright field microscope. Simultaneous viewing of both the INT-formazan crystals and the DFA-stained *Legionella* spp. by epifluorescence allows one to differentiate between actively respiring and nonrespiring cells. This technique presumes that all viable cells will be respiring, while nonviable cells will not; however, this assumption has been challenged by others who have demonstrated that some viable but nonculturable organisms do not react with INT (Roszak and Colwell 1987).

5.3.5 Effect of ATES Technology on *Legionella* spp.

The presence of *Legionella* in ATES systems will depend primarily on the ability of the organism to survive in the particular subsurface environment. With the exception of the temperature, knowledge of the physical and chemical parameters of the University of Alabama chill storage system is limited. As noted previously, *Legionella* bacteria have been isolated from many environments but they are primarily found in environments with temperatures between 25 and 45°C. In ATES systems that operate below this temperature range, *Legionella* spp. survival might be hindered. However, with recent evidence suggesting an association of amoebae and other protozoa with *Legionella* outbreaks, a mechanism for the propagation and survivability of *Legionella* can be proposed. For heat injection systems, such as the University of Minnesota system, the source water temperatures may not support survival of *Legionella* spp.

The survivability of microorganisms in the subsurface environment is also dependent on the availability of nutrients. Nutrients may be in the form of organics either generated by microflora or from chemical treatment of circulating waters; from naturally occurring compounds in the aquifer; or from inorganic compounds, such as nitrogen and phosphorus. As nutrients are depleted in a particular environment, bacteria cells will undergo changes that enable them to conserve energy and take advantage of the minimal resources available (Poindexter 1981). Decreases in size, change in shape, loss of motility, and loss of excess genetic and metabolic structures have been observed (Poindexter 1981). Starved bacteria cells have been maintained in the inactive state in

the laboratory for up to 8 months and may represent the normal state of bacteria in nature, where they apparently persist for much longer times (Amy, Pauling, and Morita 1983).

Hussong et al. (1987) showed that some *Legionella* spp. are viable but not culturable on agar media, supporting the concept that cells are able to exist in an inactive state. Loss of culturability was demonstrated with *L. pneumophila* maintained in a microcosm containing sterilized water and assayed at intervals for growth on the appropriate media and lethality to chicken embryos. The rate of decline in CFUs was temperature-dependent, with a greater decline occurring at 37°C than at 4°C. However, the DFA and acridine orange direct counts (AODC) remained stable. Upon injection into embryonated eggs, these same water samples exhibited far greater chick embryo mortality than could be accounted for by the number of culturable cells in the same samples, presumably because nonculturable *Legionella* had multiplied within embryos.

Sediment texture in unconsolidated aquifers may also influence the ability of *Legionella* spp. to survive. In groundwater systems, bacteria may collect at certain places, either through the process of adsorption or filtration and will colonize along with other microorganisms to form symbiotic communities. Adsorption processes are influenced by the chemical environment and clay content and type, while filtration depends on the flow rate of the groundwater and the pore size of the sediment in the aquifer (Gerba and Bitton 1984). Surface-associated growth in hot-water systems as well as potable water systems has been demonstrated with *Legionella* outbreaks and may account for survival of these microorganisms in ATES and cooling and heating systems.

5.3.6 Feasibility of Chemical Treatment of ATES

If further studies indicate that ATES enhances the survival of *Legionella* and other pathogenic organisms, the possibility of chemical treatment would have to be addressed. Little is known about the feasibility of chemically treating ATES systems; however, there is information on biocide treatments of cooling towers, which might be applicable to ATES. Corrosion, scaling, and biofouling are processes that could minimize heat transfer, reduce flow rates, and cause premature equipment failure in heat reinjection systems. Surveys

of various reinjection systems indicate that it is common for these systems to be contaminated with *Legionella* and other bacteria (States et al. 1987). There are several biocides (e.g., didecyldimethlyl ammonium chloride and 2,2,dibromo-3-nitril propionamide) that have been shown to be effective against *Legionella* in laboratory studies (Grace et al. 1981; Soracco et al. 1983). However, many of these biocides have been unsuccessful in environmental samples because cooling towers typically contain organic matter and are exposed to aeration and light, all of which would interfere with the activity of the biocides. In addition, *Legionella* in these environments may be protected from chemical treatment by extracellular products generated by other microflora.

Chlorination is generally the most reliable treatment for eradicating both *Legionella* and potential pathogenic organisms in aquatic systems. Levels of 2 to 3 mg Cl^- per liter water are required to achieve eradication of *Legionella* in cooling-tower waters but also may contribute to increased corrosion of the cooling towers. States and coworkers (1987) have proposed an alkaline treatment of cooling towers for control of *L. pneumophila.* Earlier studies have indicated that *Legionella* spp. are pH-sensitive and survive in a pH range of 5.5. to 8.1 in natural habitats and a pH range of 5.5 to 9.2 in laboratory studies. In their investigations, States et al. (1987) found that *L. pneumophila* multiplication was inversely correlated with pH and alkalinity. This inverse correlation with pH is consistent with field and laboratory studies conducted by Fliermans et al. (1981) and Wadowsky et al. (1985). Changes in the solution concentrations of required or toxic compounds through changes in the amount absorbed may explain the inverse correlation between survivability and alkalinity.

Approaches to controlling the survival and propagation of *Legionella* spp. in cooling towers and, possibly, in ATES-associated chill systems have been proposed. These include:

- maintain pH and alkalinity of water systems outside of the tolerance range of *Legionella* spp.
- prevent scaling and corrosion of cooling towers by the addition of organophosphorous compounds and corrosion inhibitors such as zinc and chromate

- add commercially available biodispersants to ensure *Legionella* spp. associated with biofilms are exposed to disinfectant and high alkalinity conditions
- reduce concentrations of metal, such as Mn and Zn, to levels that do not support growth of *Legionella* spp.
- maintain temperatures of water systems below 25°C or above 50°C
- select biocides that are detrimental to both *Legionella* spp. and the specific microflora found in association with this microorganism.

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