Endolith, Extremophilic Organisms from Volcanic Rock: Biochip Precursors

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Abstract

Extremophilic microbes, *Pseudomonas syzgii*, from the ultrapure water of computer circuit fabrication facilities, crystallize semiconductor matter around themselves while remaining biologically active in a novel form of biochips. The search for extremophilic organisms with similar abilities was extended to volcanic rock from "wellness" water filters. A volcanic rock material called Taicho stone was identified as harboring subterranean *Sphingomonas* species known to selectively destroy monoaromatic pollutants. They are also water-flow re-activated and productive of substantial exopolymeric substances related to the claimed multiple health-related benefits of its filtered water. Attempts to convert these *Sphingomonas* sp. from the stone host to germanium oxide crystalline host began with the filtered water aggressively corroding germanium substrata, as with *Pseudomonas syzgii*. Although semiconductors have not yet stably incorporated these biota, their roles in accelerating superficial biocorrosion suggests their additional utility as non-abrasive biochemical/ mechanical polishing aids. Recognizing that extraterrestrial exobiology has posited numerous similar microbes occluded in minerals, the possibilities for functional organism-based biochip fabrication are diverse when successful semiconductor incorporation and communication are achieved. The results suggest that the best sources for organism-based biochip production will be from endolithic media.

Keywords: biochips, oxide electronics, germanium, extremophiles, electron acceptors, biocorrosion.

1. Introduction

It has been nearly two decades since an international attempt was mounted to identify and possibly eliminate microbial contaminants in the ultra-pure rinse waters of “Fabs” (= fabrication facilities; “foundries”) for semiconductor chip manufacturing. The discovery then made of in-dwelling extremophilic microorganisms that could also corrode and embed themselves into semiconductor materials, and specifically germanium—the first transistor material[1]—launched this continuing search for other semiconductor/ bacterial adducts that could provide new generations of functional “biochips” specifically as extreme-condition sensors.[2] Figure 1 is a low-voltage scanning electron microscope image, not further coated, of one of these biochips. Exposure of such specimens to pure-water flow leads to organismal reproduction and initiation of new biocorrosion sites on germanium surfaces downstream,[2] suggesting the path to desired biochip foundries for additional extremophilic organisms now being explored with volcanic rock-derived *Sphingomonas* species.

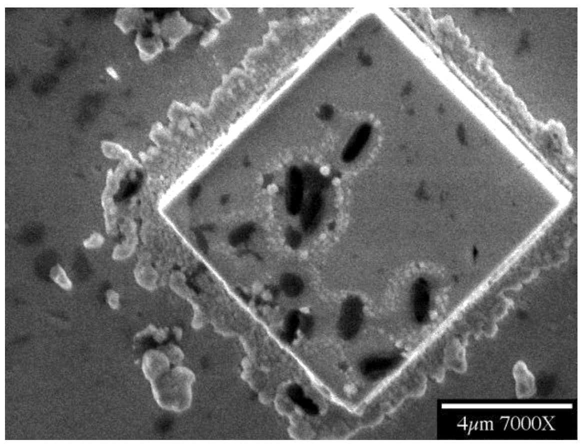

Figure 1. Scanning electron micrograph of live bacteria in GeO_2 microcrystal

The extremophile-embedded germanium oxide microcrystals so easily and abundantly produced from the Fabs’ ultrapure water supplies, and later from less-pure water sources seeded with these micro-organisms, encourages their entry into the emerging field of oxide electronics[3] where numerous similar circuitry challenges are being addressed. Recognizing that the intact biological processes of the embedded bacteria almost certainly engage electron acceptor cascades,[4] interrogation of these cascades by modulated electron flow to/from the GeO_2 chips on germanium semiconductors opens opportunities for numerous sensor and control devices. The convergence of these studies with the search for residual extraterrestrial life forms[5] is clear and influential while seeking additional test organisms.

Thus, an alternative source of potentially GeO_2-incorporable microbes has been from an anomalous behavior of certain volcanic-rock-containing water filters claiming significant health and medically-related benefits for its “purified” water,[6] from which 16SrRNA genes identified active *Sphingomonas* species *S. subterranean* (AB025014) and S. *aromaticivorans* (U20774). The feed water to the filter unit, according to similar

16SrRNA analyses, showed only *Acinetobacter* sp. ATCC17925 (accession number Z93441).

2. Methods and Materials

The major material of analysis was crushed volcanic rock (called Taicho stone), of light green and pink hues, from Central Japanese volcanoes,[7] pictured spread over a germanium prism in the Figure 2 scanning electron micrograph.

Germanium prisms were key materials for both their substance and infrared energy transmission capabilities throughout this work, allowing a multiplicity of analyses[8] while serving simultaneously as the corroding substratum expected to incorporate extremophilic microbes in their re-crystallizing superficial oxides. An internal reflection infrared[9] spectrum of a Taicho stone smear on a Ge test plate is given as Figure 3, and an energy-dispersive X-ray spectrum is provided as Figure 4. These data illustrate the starting qualities of the Taicho stone to be a majority aluminosilicate mineral having minor iron and potassium contents, with trace hydrocarbon matter present.

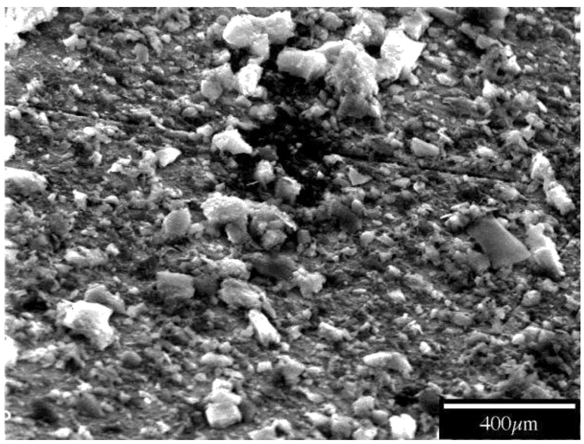

Figure 2. Taicho stone from water filter, spread over germanium surface

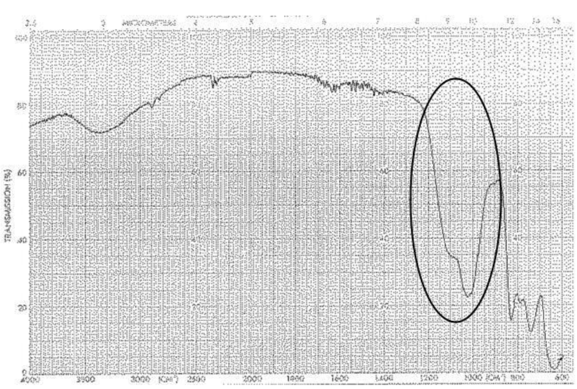

Figure 3. Internal reflection IR Spectrum of Taicho stone on Ge in Figure 2 (IR absorptions from stone are circled)

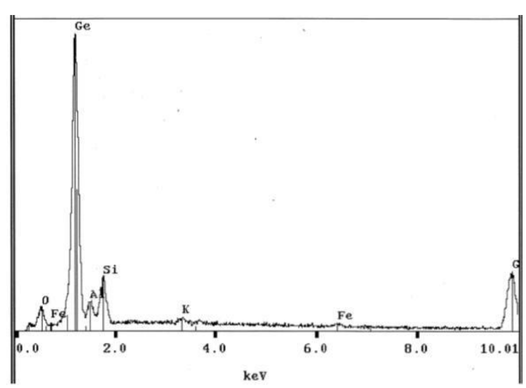

Figure 4. EDX-ray elemental analysis of Taicho stone on Ge in Figure 2

Water contact angle measurements on reference-grade Teflon film[8,10] were used to ascertain whether the filtration material added surface-active constituents to the water. In this test, pure distilled water gives an average measurement of approximately 120 degrees. In the experiments, both tap water and distilled water passed through the filtration device that did not contain Taicho stone showed contact angles of between 113 degrees and 119 degrees on the Teflon, the higher values after filtration. In contrast, the same water sources passed through the filtration device containing the Taicho stone exhibited contact angles of only 107-109 degrees on the Teflon. This surface tension depression of water indicated a specific elution from the Taicho stone of some surface-active, surface-tension-lowering material consistent with the trace organic matter noted in the infrared spectrum (note small absorption bands at 2850 and 2950cm^{-1} in Figure 3).

For longer-duration experiments, analytical flow cells[11] (each containing two clean, smooth Ge internal reflection trapezoidal test plates; 20x50x1mm) conducted tap water through 13x50x1mm rectangular channels at 350 ml/min (10 dynes/cm^2, 1 Pa) for increasing times before the Ge test plates were reanalyzed by infrared spectroscopy and re-inspected by scanning electron microscopy and EDX-ray techniques. Operationally, then, it was arranged that side-by-side filtration units, one without Taicho stone and one with Taicho stone operated identically for long periods of time before inspecting the germanium prisms' flow surfaces both spectroscopically and microscopically.

3. Results

As shown in Figure 5, equal exposure of clean Ge prisms to filtered water flow with and without Taicho stone in the filter device showed dramatically different results. It is also noteworthy that the presence of the Taicho stone correlated with a much-more-rapid, obviously biocorrosion-accelerated, rate of superficial erosion of the germanium prisms.

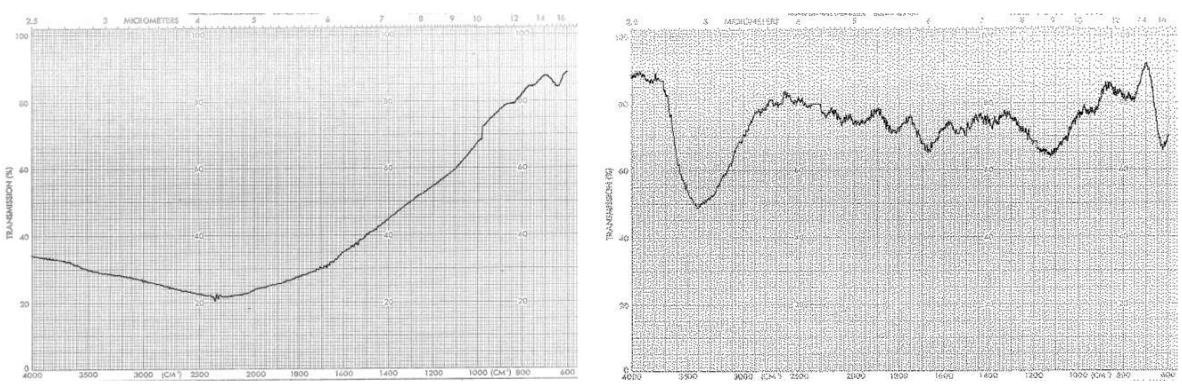

Figure 5. Transmission IR spectra comparing water deposits on Ge test plates after water passage through filter devices without (left) and with (right) Taicho stone.

The water control (no exposure to Taicho stone) flowing over germanium in its most corrosive state left a superficially corroded Ge surface bearing no evidence of organic matter. In contrast, that same corrosive stream passing over Taicho stone did acquire and subsequently deposit onto the Ge surfaces an increasingly dense deposit of biofilm material that actually diminished the corrosion. The kinetics of this process are compared in the internal reflection infrared spectra of the filter effluent (Figure 6). An abundant "slime" deposit formed on the Taicho stone and released glycoproteinaceous components into the water.[12]

As revealed in Figure 7, however, the incorporation of the living, slime-producing extremophilic *Sphingomonas* organisms was not retained in regular GeO_2 microcrystals over the corroding Ge surface. This feature is likely to require careful controls of both water velocity and purity, as already observed for the *Pseudomonas syzgii* organisms.[2] Figure 8 does reveal trace carbon and phosphorous components to have been present in the Taicho stone-contributed surface layers on the germanium surface early in the experiment.

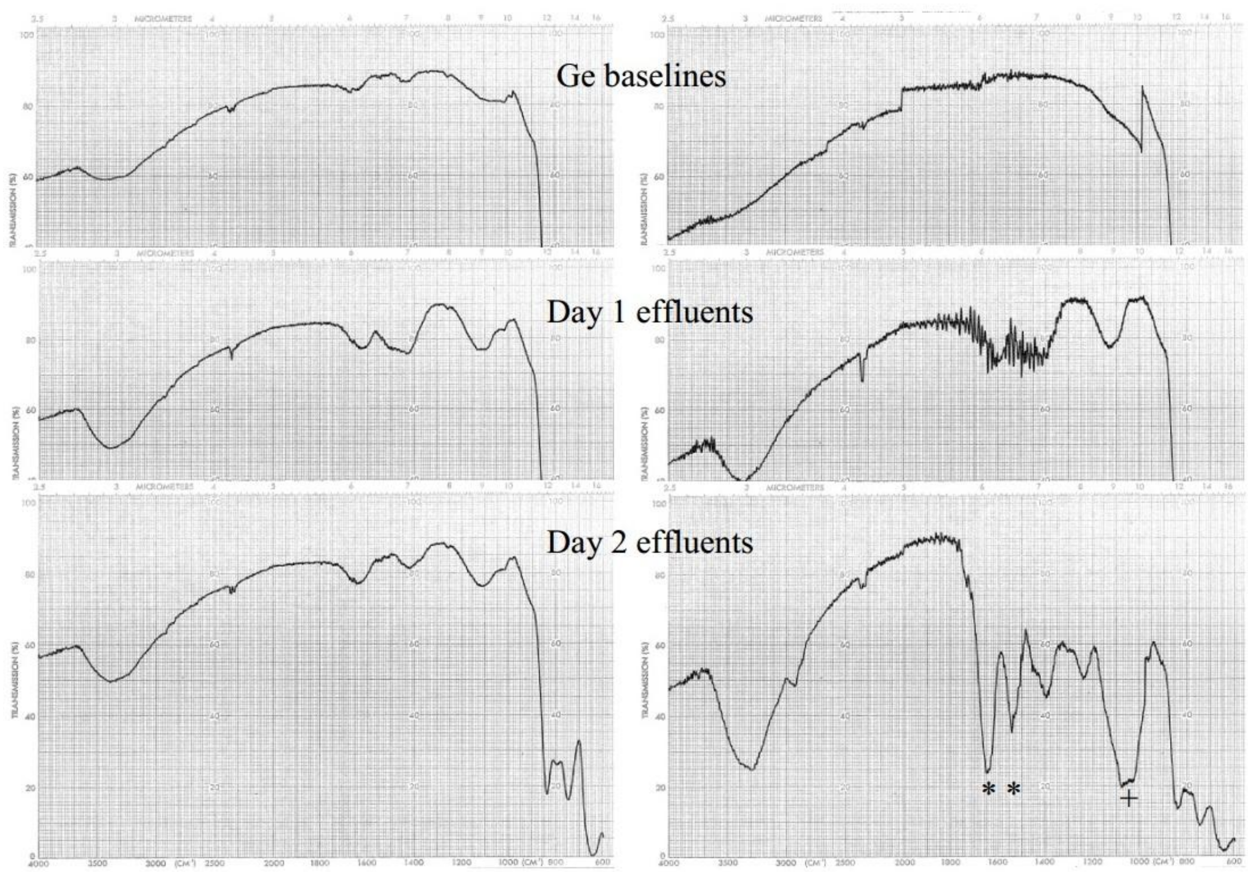

Figure 6. IR spectra of effluents from devices without (left) and with (right) Taicho stone. Controlled volumes of the effluents were evaporated on Ge test plates prior to analysis. Note glycoproteinaceous character of Taicho stone slime effluent (* Amide I & II, + carbohydrate).

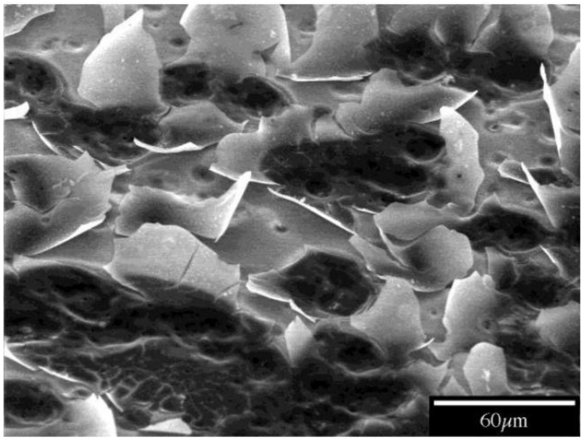

Figure 7. Biocorroded Ge surface; not yet crystallized

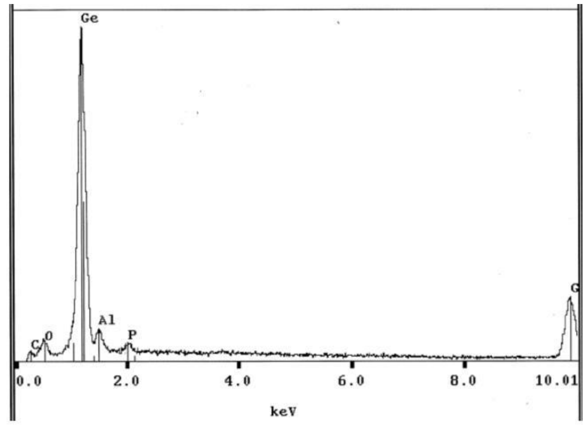

Figure 8. EDX-ray elemental analysis of initial deposit (note trace carbon and phosphorous components)

4. Discussion

4.1. The Water Supply

Numerous beneficial but peculiar features have been anecdotally claimed in popular, consumer literature for water produced by "wellness" filter units (e.g. better plant growth, smoother hair, elimination of skin rashes, greater solubilities, and more), drawing attention to the filter beds. Especially interesting were reports that these properties all improved with water-flow duration. Thus, water flows through each and then all of the filter beds' components were examined, over increasing times. All but the final filter layer behaved conventionally to produce effluent that was organic-free and of slightly higher surface tension than its feed supply waters. The final filter layer was a crushed volcanic rock called Taicho stone having pink or green hues.

Unlike the other filter components, the layer of Taicho stone in contact with the filtered water caused that water to diminish in surface tension in a manner suggesting addition of organic surface-active ingredients. With increasing flow durations, the filter effluent was shown to become increasingly enriched in slime-producing matter having the characteristics of a glycoproteinaceous biofilm long associated with *Sphingomonas* microorganisms.[12] Samples of the emerging water revealed that extremophilic *Sphingomonas* species had become active and both they and their metabolic products had "fertilized" the waters. Analyses by genetic 16SsRNA methods indicated their best homology was with *S. aromaticivorans,* known to be a potent reducer of groundwater pollutants,[13,14] and *S. subterranean* as often found in deep ground layers.[15] Prior to release of the glycoproteinaceous slime from the organisms, the initial superficial corrosion of clean, smooth germanium over which this water flowed was even more aggressive than that of the control water passing through Taicho stone-free filters. Thus, these effluent waters were tested in the flow cell model that had produced the pioneer biochips from similarly corrosive water sources.[2]

4.2. Rock-encased Organisms

Amazing micro-Computerized Tomographic (μCT) images of microbes encased in sedimentary rocks have been shown,[16] and confirmed by conventional transmission electron microscopy. Many prior reports exist about the microbial diversity and abundance of subterranean[17,18] (and possibly extraterrestrial[19]) extremophilic organisms. Thus, there is a clear understanding that there can actually be "life in rock" of a considerable range.[20] However, until our prior discovery that these microbes could be subsequently transferred to and encased within microcrystals of solid-state germanium (reminiscent of the first invented transistors), this intriguing new definition for true organism-based biochips wherein electronic flow-modulated information transfer might occur was not anticipated. What has the Taicho stone now added to these prospects?

4.3. Taicho Stone Origins and Emergent, Functioning Bacteria

Taicho stone is claimed to be a rare volcanic (igneous) mineral substance formed by the cooling and crystallization of molten volcanic rock between 1100F and 2200F temperature and from ground level to depths greater than 98,000 feet. It is apparently named after a sixth century (CE) Nara period monk in Japan, said to be the first person to reach the top of Mount Haku in the Ryohaku mountains.[7,21] It has been employed since 1984 in so-called "wellness filters" endorsed by the Kitazato Medical School of Public Health in Tokyo and since 1995 by the Japanese Ministry of Health. The filters have been installed in numerous hospitals as well as in NEC Corporation office buildings throughout Japan since 2000. It was shown to produce filtered water with both a lowered surface tension and increased solubilization capability.[6,22]

Although volcanoes first erupt as the Earth's tectonic plates diverge, with fluid molten rock (magma) flows arising from the voids between the plates, geothermal water that originates from cold rainwater does seep through the resulting crust. Nevertheless, any originally entrapped organisms must truly be in the extremophile category because water temperatures of 150-200C have been reported in 1000m-deep fields across volcanic belts. It is clear that, whenever they entered the rock, the *Sphingomonas* species are capable of re-activation by clear water flows. Carbon dioxide outgassing during volcanic events may have entrapped sufficient carbon to sustain minimal metabolic events. It is speculated that the source of organic carbon might have been lignite from plant material buried within the rock,[23] both the gaseous and organic sources being coupled to reduction of electron acceptors. Via such properties, members of the *Sphingomonas* genus are well-recognized for their degradation of many exotic organic compounds of aromatic and halogenated chemistry.[13,23] Their use in bioremediation of groundwater pollutants is well-known,[14] as is their isolation from Finnish and Swedish drinking water distribution systems,[15] which often employ selective growth media.[24] With regard to their provision of special features that may be encapsulated in semiconductor oxides, it has already been noted that the exploitation of *Sphingomonas* sp. metabolic capabilities could provide important biotechnological benefits.[25]

4.4. Oxide Electronics

It is clearly established that electron acceptor features at or within oxide electronic materials are especially important within the "roadmap" to important new generations of sensors and switches.[3,26] In a novel approach to describe primary olfaction, electron tunneling of odorant molecules is invoked, and electron tunnel spectroscopy is proposed for sensing various molecules[27] that might be especially important in military challenges. In the context of semiconductors, their ability for electron flow to be easily modulated might be exploited by biological features of embedded functional microbes. The under-utilized but extraordinarily sensitive "contact potential" method could be re-visited to detect and analyze polarization features of adsorbed liquids[28,29] or vapors,[30] thereby providing a hardy electronic method of detecting impurities in the air. Application of this method to monomolecular organic films on the same types of germanium prisms used here and in previous extremophile work[2] has already shown microvolt sensitivity to configuration changes of model proteins.[31] Can these findings be relevant to oxide electronic features of the same materials bearing corrosion-induced entrapped extremophiles?

Perhaps more immediately, successes with luminescence measurements of extraordinary "molecular thermometers" for measurements at the microcrystal scales are now producible and might be exploited.[32,33]

4.5. Extremophilic Organisms

It is intriguing that these studies are also entwined with curiosities about extraterrestrial life. For example, specific to the volcanic origin of microorganisms described here, two species of *Picrophilius* microbes isolated from volcanically heated, dry soils in Japan[5] should be tested for their amenability to GeO_2 encapsulation while retaining their acid-loving and thermophilic qualities.

Especially important to note is that substrata for endolithic life have already been explored in the search for life on Mars, examining further the rocks from pyroplastic flows of volcanoes, which deposits cover large areas of that planet's surface. Small chips of such rock have shown a green-colored horizon beneath the rock surfaces (similar to Taicho stone) and also illustrate life-describing biological features and morphologies when suspended in distilled water.[19] Can this entrapment of extremophilic life forms be converted to similar entrapment in functional semiconductor oxides by the corrosion mechanism?

4.6. Biocorrosion Benefits

In the course of these investigations, it was found that the microbe-induced corrosion of germanium substrata was at a dramatically enhanced rate (prior to slime formation) when compared to even microbe-free ultrapure water, suggesting a new method of "biochemical-mechanical" planarization of semiconductors,[34] without the negative consequences of abrasion from currently used oxide slurries. This technical use, by itself, could have significant commercial and military benefits.

5. Conclusion

Extremophilic organisms released into flowing pure water are capable of aggressively corroding the surface of pure germanium. In one prior example[2], so-entrapping those reproducing microbes into GeO_2 "biochips" suggested the process here partially reproduced with another emergent extremophile and setting the stage for future "foundries" of true Biochips. Endolithic extremophiles are the best probable future source from which the microbes will be selected.

Remaining studies must address the rates of water flow over the corroding surfaces, and the water purity levels necessary to produce abundant, functional microcrystals as done previously[2] for organisms from chip-fabrication facility waters. Interrogation of these new manufactured Biochips will utilize the emerging techniques of oxide electronics[26], and usher in an extraordinarily sensitive generation of functional biotransistors for use in medicine, commerce, and military settings.

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