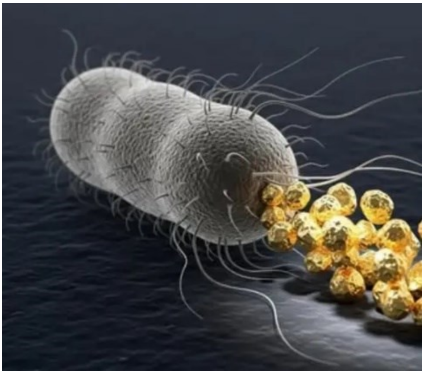
Bioreactor

How to make your own gold



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Forward

Most literature and scientists state that there is a bacteria that does make gold as it processes toxic compounds into 24-karat gold nanoparticles, but that it is not a viable "gold-making opportunity" for investors or commercial-scale production. They say the process is an example of "microbial alchemy" that offers potential for eco-friendly gold recovery in the distant future, but it is not a realistic get-rich-quick scheme. They further state that the endeavor is flawed because it requires gold as an input to the process; that it is impractical to make it a large-scale operation; and that the amount of gold produced by a single bacterium is microscopic and that collecting it would be an enormous and costly undertaking.

They are all so wrong.

Chapter 1: Growing Gold with Bacteria

The bacteria known as Cupriavidus metallidurans can transform toxic heavy metal compounds into pure, gold nanoparticles as a defense mechanism against poisoning. These bacteria live in metal-rich environments and, when exposed to mobile gold ions from decaying ores, activate enzymes that convert these harmful compounds into harmless, inert gold particles. This process creates microscopic gold nuggets and plays a role in the biogeochemical cycle of gold, turning it from a mobile, toxic form back into a stable, metallic form.

Cupriavidus metallidurans thrives in environments rich in toxic heavy metals, such as metal-contaminated soils, mine waste, and industrial sites, where it utilizes its remarkable metal resistance mechanisms to detoxify pollutants and produce metal nanoparticles of gold. This metallophilic bacterium can also grow under anaerobic conditions and chemolithoautotrophically on hydrogen and carbon dioxide, demonstrating resilience even in nutrient-poor and low-oxygen environments.

Metal-rich soils:

This includes naturally mineralized zones, auriferous (gold-rich) soils, and "zinc deserts" with high concentrations of toxic metals.

Industrial sites and wastewater:

It colonizes contaminated industrial sites, wastewater treatment plants, and groundwater remediation systems, where it plays a key role in bioremediation.

Biofilms:

C. metallidurans forms biofilms on soil particles and other surfaces, which helps it to cope with adverse conditions such as starvation and fluctuating pH and temperature.

Heavy metal resistance:

The bacterium possesses genes on large plasmids that enable it to detoxify heavy metals through mechanisms like complexation, efflux, and reductive precipitation.

Facultative anaerobic life:

It can survive and function in environments both with and without oxygen, and can also grow by converting hydrogen and carbon dioxide into organic compounds.

Chemolithoautotrophic capabilities:

Many strains can survive in nutrient-poor conditions by using hydrogen and carbon dioxide as their energy and carbon sources.

Biomineralization:

C. metallidurans is capable of turning toxic metal complexes, like gold chloride, into inert metal nanoparticles of gold, which it excretes as a byproduct.

Bioremediation:

Its ability to remove and detoxify heavy metals makes it a valuable tool for cleaning up polluted sites and recovering precious metals from electronic and other waste streams.

Gold formation:

By forming gold nanoparticles, C. metallidurans plays an active role in the formation of secondary gold deposits in nature.

It has been observed in laboratory settings that Cupriavidus metallidurans can convert toxic gold compounds into pure, 24-karat gold within approximately one week. This timeframe refers to the transformation of soluble gold chloride into tiny, solid gold nanoparticles.

The process is part of the bacteria's detoxification mechanism. In order to survive the toxic gold compounds in their environment, the microbes convert them into a less toxic, metallic form.

Factors influencing the conversion speed

While a week is a general estimate observed in some lab studies, the actual rate of gold biomineralization is dependent on several factors:

Presence of copper: For C. metallidurans, gold toxicity is linked to copper regulation. The presence of copper can inhibit the bacteria's ability to excrete copper, which in turn leads to the production of gold as a detoxifying reaction.

Metabolic activity: The conversion is not a passive event but an active metabolic process. Experiments with metabolically inactive cells do not show the same gold accumulation.

Environmental conditions: Conditions such as pH and the concentration of gold compounds can also affect the speed and efficiency of the process.

Cupriavidus metallidurans generally thrives in a neutral to slightly acidic pH range, with an optimal pH often cited as 7.0, or slightly lower depending on the specific strain and growth conditions.

Key factors concerning the pH needs of this bacteria include:

Optimal growth at pH 7.0: Laboratory studies have shown that the highest numbers of viable cells and greatest metabolic activity occur around a neutral pH of 7.0.

Tolerance of acidic conditions: While it prefers a neutral pH, C. metallidurans demonstrates a tolerance for acidic environments. For example, some strains can function in pH as low as 5.0. One study noted that a closely related strain, Cupriavidus sp. YNS-85, effectively degraded pentachloronitrobenzene under acidic conditions.

Metal immobilization in low pH: Some strains of C. metallidurans can immobilize toxic metals like uranium even at extremely low pH values (pH 1.0) by using a passive biosorption process, though active growth ceases in such highly acidic conditions.

Adaptation and buffering: The bacteria can influence the surrounding pH in certain conditions. For instance, when grown in the rhizosphere of plants, C. metallidurans has been observed to decrease the pH of the soil. In gold biomineralization experiments, the final pH of the culture tended to approximate 7.0, even when the starting pH varied.

In summary, Cupriavidus metallidurans is highly adaptable and can survive in a range of conditions, but its maximum metabolic activity and best growth occur around a neutral pH of 7.0.

Bacterial population: The amount of gold produced is directly related to the size and activity of the bacterial colony.

Environmental conditions

Temperature: Maintain a constant temperature of 30 °C (86 F) for optimal growth. Higher temperatures, such as 37°C (98 F), can be lethal for many strains and induce mutations, a phenomenon called "temperature-induced mortality and mutagenesis" (TIMM). Low temperature survivability is good at 7.8 ± 3.9 °C (40 F).

Oxygen: C. metallidurans requires aerobic conditions. For large volumes, use a shaking incubator or a bioreactor with aeration to ensure the culture is well-oxygenated.

pH: The optimal pH for growth is around 7.0. The Tris buffer in MM284 medium helps maintain this stable pH. Grow the bacteria in a Tris-buffered mineral medium (MM284) that provides specific salts and a carbon source. A standard recipe includes Tris/HCl, sodium chloride, potassium chloride, ammonium chloride, various other salts, and a source of iron, zinc, manganese, cobalt, and copper.

Large-scale cultivation in a fermenter (bioreactor)

For "huge" colonies, a bioreactor is the necessary next step. While research literature describes laboratory cultivation methods, industrial-scale high-density growth requires specialized strategies.

Prepare the inoculum:

Start a small culture in a flask using a routine growth method.

Culture for 24–48 hours until you reach a mid-exponential growth phase, with an optical density (OD600) of around 0.6.

Use this dense, healthy culture to seed the larger bioreactor to ensure a strong start and minimize the lag phase.

Use a fed-batch strategy:

This technique is key to achieving high cell densities. Instead of adding all nutrients at once (batch culture), a fed-batch system involves the controlled, continuous addition of a nutrient source, like gluconate.

This prevents the buildup of inhibitory byproducts and allows for maximum biomass accumulation.

Controlling the feed rate is important; a well-controlled, continuous feed has been shown to be superior to pulse feeding for high biomass production in related bacteria.

Optimize aeration and mixing:

Maintain high aeration and continuous mixing to ensure uniform distribution of nutrients and oxygen.

In a bioreactor, parameters like stirring speed (RPM) and air flow can be adjusted to meet the high oxygen demand of a dense culture.

Grow the bacteria in dark conditions.

Challenges with large-scale cultivation:

pH fluctuations: With high cell densities, metabolism can cause pH shifts. Using a buffered medium (like MM284) and monitoring the pH with a bioreactor probe is essential.

Nutrient limitation: Insufficient feeding can lead to nutrient-limited conditions, causing growth to plateau. A fed-batch strategy with real-time monitoring can prevent this.

Byproduct inhibition: At high concentrations, metabolic byproducts can inhibit growth. Fed-batch cultivation helps to avoid this by not overloading the system with carbon sources.

Plasmid stability: For engineered strains, managing genetic stability can be a concern over many generations. Systems like post-segregational killing (PSK) can be employed to maintain plasmid stability in related organisms during bioreactor cultivation.

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