Microbial Induced Crystallization Technology Ushering New Hope for Removing Phosphorus from Wastewater

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Abstract. In recent years, the eutrophication of water bodies in China is serious, and the available phosphorus resources are depleted. To achieve simultaneous phosphorus removal and phosphorus recovery in sewage has become a hot issue. However, there are still many problems with the existing phosphorus removal technologies. Microbial induced crystallization technology (MICT) has been applied in various fields with its unique advantages, and it also has been applied to the removal of phosphorus from wastewater. In order to provide a new idea for phosphorus removal, the currently widely used technology of phosphorus removal from wastewater and its existing defects were introduced firstly. Then a new type of wastewater removal technology - microbial induced crystallization technology and its development status and existing problems were summarized. Finally, the limiting factors of microbial induced crystallization techniques were provided.

1. Introduction
With the development of country and the continuous growth of industrial enterprises, more and more high-concentration wastewater was discharged into natural water bodies, which results in the proliferation of algae in the water, the lack of dissolved oxygen in water, and the serious eutrophication of water bodies. And the phosphorus in water is a key factor in this phenomenon [1]. According to reports, when the total phosphorus content in the water reached 0.015 mg/l, it would be sufficient to cause eutrophication of the water [2]. Therefore, how to remove phosphorus from wastewater has become an urgent problem to be solved.

At the same time, phosphorus is the trace element that all life (such as animals, plants, and bacteria) rely on to survive, and is an indispensable nutrient for crops to be produced at high yields [3]. However, in the earth element cycle, the phosphate resources are renewable and cannot be recycled. Especially, China’s phosphate resources are endangered and listed as one of the major mineral resources that cannot meet the needs of the country’s economic development [4].

Therefore, how to achieve efficient removal of phosphorus in wastewater and then recycle it to supplement the near-exhausted phosphorous resources has become a research hotspot. In order to provide readers with a comprehensive understanding of China’s current wastewater removal technology, the following article will detailed introduce the traditional phosphorus removal technology and the new phosphorus removal technology respectively.
2. The Traditional Phosphorus Removal Technology

2.1. Status
The traditional phosphorus removal methods for wastewater mainly include biological, chemical, and crystallization methods [5]. Next, we will introduce the principle of phosphorus removal and the defects in these methods, as follows.

The biological method mainly uses polyphosphate accumulating organisms (PAOs) to remove phosphorus. The PAOs absorb volatile fatty acids (VFAs) stored in the solution under anaerobic condition as intracellular polymer (poly-β-hydroxybutyrate, PHB) and release phosphorus simultaneously. Under the subsequent aerobic condition, PAOs takes oxygen as an electron acceptor, uses PHB stored in vivo to obtain adenosine triphosphate (ATP), absorbs excess phosphorus in solution and stores it as polyphosphate particles [6]. Finally, the phosphorus removal from the wastewater is achieved by discharging excess sludge [7].

The chemical method mainly uses chemical precipitant to convert the phosphate into insoluble precipitate, and then achieves the phosphorus removal of wastewater through the physical precipitation [8]. At the same time, the formed floc also has adsorption and removal effect on phosphorus [9].

The crystallization method mainly by adding phosphate, ammonium salt or calcium and magnesium ions into the ammonia nitrogen rich or phosphorus rich wastewater, and then controlling the ion state in the solution by adjusting the pH value, the crystal species, the ultrasonic, to achieve the phosphorus removal [10]. The main crystalline product is magnesium ammonium phosphate (MAP) or hydroxyapatite (HAP), which is difficult to dissolve in water [11]. Finally, the removal of phosphorus is achieved by recovering the crystallization of phosphorus compounds.

2.2. Problems
However, when the anaerobic digestion method is used to treat the phosphorus-rich excess sludge produced by the biological method, the digested sludge supernatant and the dehydrated filtrate are difficult to handle, and the phosphorus cannot be recovered and reused [12]. Similar to the biological method, the chemical method can only remove phosphorus but can't recover it [13]. Because the resulting amorphous metal phosphate precipitate cannot be separated from the biological sludge [14]. For the crystallization method, because the phosphorus concentration in the domestic sewage is too low to naturally form crystals, it is necessary to strictly control the crystallization conditions to achieve high-efficiency phosphorus removal. So the operation is difficult.

Therefore, finding a more suitable method for removing phosphorus from wastewater has become an urgent problem to be solved. In recent years, it has been found that some unicellular microorganisms can use their own physiological and biochemical characteristics to induce the formation of crystals and thus meet the needs of various practical projects such as removal of various pollutants [15] and soil solidification [16]. Since then, a large number of researchers have been conducting related research and found that under certain circumstances, microorganisms can be induced to form phosphorus crystals [17]. In nature, the presence of microbial induced crystallization is very common [18]. Thereby, this article will mainly introduce this technique.

3. Microbial Induced Crystallization Technology

3.1. Overview

3.1.1. Principle. Microbial induced crystallization is that certain microorganisms promote crystallization by secreting metabolites under certain conditions, or use the soma body as crystal nuclei to induce the occurrence of crystallization according to their specific cell structure, as shown in Figure 1.
3.1.2. Advantages. The crystallization is induced by microorganism's own metabolic product or its own physiological function, with low investment and low cost. No chemical agents are required to promote the production of crystals, so no byproducts appear, and no pollution is caused to the environment. The remains or activated sludge produced by microorganisms can be used as feed and fertilizer for the recycling of resources because they contain a large amount of protein and nutrient elements.

![Figure 1](image)

**Figure 1.** The principle of microbial induced crystallization. (a) The ions A secreted by the microorganism itself binds with the ions B in the environment to form insoluble precipitates; (b) Some microorganisms are negatively charged, which can attract positive ions in the environment to form insoluble precipitates.

3.2. Application

The research on MICT can be traced back to the early last century [19]. It has been applied to many fields such as building materials, heavy metal fixation and recycling, and wastewater treatment.

3.2.1. Microbial Induction Crystallization of Reinforcement. Boquet [20] believed calcite crystal produced by soil bacteria was a common manifestation. For this purpose, the author isolated 210 crystal-forming organisms on B-4 solid medium, and all bacteria isolated from the soil were included, which can conclude that crystal formation is a common phenomenon and is only related to the organic components contained in the medium. Zhang [21] used lysophila which called *Bacillus paenibacillus* to treat liquefied sand. The mechanical properties of the treated sandy soil were tested by dynamic tests. The results showed that microorganisms can use the urease produced in the metabolic process to induce precipitation of calcium carbonate, thereby improving the liquefaction resistance of the sand. It was confirmed that the microbial grouting technique can greatly improve the liquefaction resistance of sand. The reaction process is shown in Figure 1. Shao [22] used a layer of microbial mortar to protect the surface of the silt, and analysed the influence rules between the thickness and strength of the microbial mortar protective layer. The results showed that the microbial mortar layer has good erosion resistance and can be used for silt surface protection. Calcite crystals induced by microorganism can form a cemented microstructure that wraps around the sand particles, enabling the surface protection mortar layer to have good resistance to water flow erosion.
3.2.2. Microbial Induced Crystallization of Phosphorus Removal. The studies have found that some unicellular microbial cell bodies can act as seed crystals and induce the formation of phosphorus crystals on the cell surface. Sun [23] used a high-efficiency phosphorus removal bacteria separated and screened from deep-sea sediments combined with steel slag to treat high-salt synthetic wastewater with high phosphorus concentrations. Scanning electron microscopy and atomic force microscopy observations revealed that crystals and organometallic phosphorus precipitates formed on the surface of bacteria. Infrared spectroscopy revealed that the bacteria combined P with surface functional groups to form P-O-C bonds for phosphorus removal. It was concluded that the bacteria had a role in inducing metal phosphate precipitation. Wang [24] speculated the mechanism of the extracellular polymeric substances (EPS) secreted by marine bacteria CF8-6 in the phosphorus removal process of strains, and verified it by means of morphological and compositional characterization methods such as atomic force microscopy. The results showed that during the process of phosphorus removal, a large amount of particles were produced outside the cell, and the particles contained a small amount of metal ions such as Ca, Mg, etc. Therefore, CF8-6 can induce the metal ions and phosphorus in the solution to form metal phosphate precipitation to achieve the goal of phosphorus removal. EPS can remove phosphorus by trapping phosphorus and forming extracellular organic phosphorus particles. Phosphorus in the particles is mainly linked to polysaccharides. The main component is phosphorus ester. Qiu [25] isolated and purified the microbial population of the A/O alternating biofilm system and found a strain of yeast. When inoculated this yeast strain into a medium containing magnesium salts and cultured it, there will be some cake-like, spherical and hemispherical magnesium ammonium phosphate crystals produced on the surface of the yeast and around it, which exhibited a pronounced phosphorus crystal-induced induction effect.

3.3. Problems
MICT has attracted the attention of more and more scholars because of its unique advantages. However, there are also many problems that limit the wide application of this technology.

1. Microorganisms’ metabolic activity is affected by external conditions such as temperature, pH and substrate concentration.

2. The environmental adaptability is poor, which could result in unstable crystal formation and failure to maintain the different needs of humans in an efficient state.

3. The number of microorganisms currently present is huge. How to find out which kind of microorganism is you need among a large number of microorganisms, requires to read a wide range of literature and to do a lot of experiments. Therefore, the energy input in the early period is large.

4. Concluding Remark
At present, the phenomenon that microorganisms can induce crystallization has been widely recognized. The MICT has been popularized and used in various fields, and has also obtained very good results in the field of phosphorus removal from wastewater. However, the current MICT only uses the original physiological and biochemical characteristics of the microorganism to achieve quantitative phosphorus removal. It is guessed whether it is possible to change the metabolic...
properties of microorganisms through special techniques to obtain higher phosphorus-inducing effects of microorganisms. I think if this conjecture is feasible, a great leap will be obtained in the field of sewage phosphorus removal. Therefore, this subject deserves further study.

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