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เรื่อง ขออนุมัติค่าตอบแทนการตีพิมพ์ในวารสารวิชาการระดับนานาชาติ

เรียน รองคณบดีฝ่ายวิจัยและวิชาการ ผ่านหัวหน้าภาควิชาวิศวกรรมเคมี

อ้างถึงประกาศฉบับที่ 40/2550 ประกาศ ณ วันที่ 22 ตุลาคม 2550 คณะวิศวกรรมศาสตร์ เรื่อง "หลักเกณฑ์การจ่ายค่าตอบแทนการตีพิมพ์ผลงานวารสารวิชาการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี" ตามความทราบแล้วนั้น

เนื่องด้วยดิฉัน นางสาวสุมนา สิริพัฒนากุล อาจารย์ประจำภาควิชาวิศวกรรมเคมี ได้ตีพิมพ์บทความวิชาการ เรื่อง "Fundamentals and Applications of Entrapped Cell Bioaugmentation for Contaminant Removal" ในวารสารระดับนานาชาติ Emerging Environmental Technologies Volume II pg. 174-169 โดยมีสัดส่วนการทำงานคิดเป็นร้อยละ 70 ดังนั้นดิฉันจึงใคร่ขออนุมัติค่าตอบแทนการตีพิมพ์ผลงานดังกล่าว

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แบบแสดงหลักฐานการมีส่วนร่วมในการเขียนบทความวิชาการ

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Chapter 7
Fundamentals and Applications of Entrapped Cell Bioaugmentation for Contaminant Removal

Sumana Siripattanakul and Eakalak Khan

Abstract Entrapped cell bioaugmentation is an addition of gel or rubber matrices embedded with microorganisms to increase biological activities. The technology is an integration of cell entrapment and cell bioaugmentation techniques. In the last decade, this technology has been frequently studied for its applications in the environmental field for removing collective and specific contaminants. The technology not only provides sufficient contaminant-degrading cultures but also prevents them from environmental stresses and being transported out of the target systems. This paper provides a review on the uses of entrapped cell bioaugmentation for contaminant removal including background of the technology, principles of cell entrapment techniques, types and preparation procedures of selected cell entrapment matrices, and studies on the applications of the technology for wastewater treatment and site remediation. Future perspectives of the technology are also discussed.

Keywords Bioaugmentation · Biodegradation · Bioremediation · Cell entrapment · Wastewater treatment

7.1 Introduction

Engineered and natural biological processes sometimes do not perform well or take long time in removing contaminants such as nutrients, heavy metals, phenolic compounds, and chlorinated compounds because they have inappropriate types and/or insufficient numbers of contaminant-degrading cultures [1–7]. A technique called cell bioaugmentation, was developed to overcome these problems [1–9]. Cell bioaugmentation is the addition of adequate numbers of effective contaminant-degrading microbial strain(s) to remove contaminants. The cell bioaugmentation

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technique has been applied to remove several contaminants, such as 3-chloroaniline, 2,4-dichlorophenoxyacetic acid, and 3-chlorobenzoate, in wastewater and contaminated sites [2, 3].

The key attributes for the success of cell bioaugmentation are the viability and retention of the bioaugmented cells in the target systems [2, 4, 7, 10]. In field applications, the augmented cells might experience biotic and abiotic environmental stresses, such as predation and competition with indigenous species and presence of inhibiting compounds [11]. Moreover, the augmented cells sometimes leave the systems along with the effluent or groundwater flow for the cases of wastewater treatment or site remediation, respectively.

Cell entrapment, a cell immobilization method by embedding microorganisms in a porous polymeric matrix, can be used to alleviate the shortcomings associated with the traditional planktonic (suspended or free) cell bioaugmentation scheme. Some of the common natural and synthetic polymeric materials used as cell entrapment matrices include calcium alginate (CA), carrageenan (CN), polyvinyl alcohol (PVA), and cellulose triacetate (CTA). Cell entrapment has been studied and applied mainly as stand-alone wastewater treatment processes for the removal of collective pollutants, such as organic carbon [12–20] and nitrogen [21–27], as well as specific contaminants such as phenol [1], dyes [28–31], and cyanide [32]. Recently, the technique was combined with cell bioaugmentation resulting in a new process, known as entrapped cell bioaugmentation, for removing pollutants, such as nitrogen, herbicide, and other hazardous compounds in wastewater and contaminated sites [6, 7, 33, 34]. The entrapment matrix can protect the augmented cells against environmental stresses and prevent their loss from the target systems making entrapped cell bioaugmentation a more reliable technology compared to the traditional planktonic cell bioaugmentation.

This article reviews the basics and applications of entrapped cell bioaugmentation for contaminant removal. The principles of cell entrapment, types and preparation procedures of selected cell entrapment matrices including CA, CN, PVA, and CTA, and advantages and drawbacks of entrapped cells compared to suspended or free cells are described. Previous studies on the applications of entrapped cell bioaugmentation for wastewater treatment and site remediation including success, concerns, and future perspectives of the technology are also discussed.

7.2 Cell Entrapment

Entrapment is one of the cell immobilization techniques in which microorganisms are embedded within porous polymeric supporting materials (Fig. 7.1f) [35–37]. Some other common cell immobilization techniques include physical adsorption, ionic binding, covalent binding, cross-linking, and encapsulation (Fig. 7.1a–e). In entrapment and encapsulation, microorganisms are not directly bonded but enclosed in supporting porous matrices. The cell entrapment technique confines microbial cells within the pores and voids of immobilization matrix while the cell

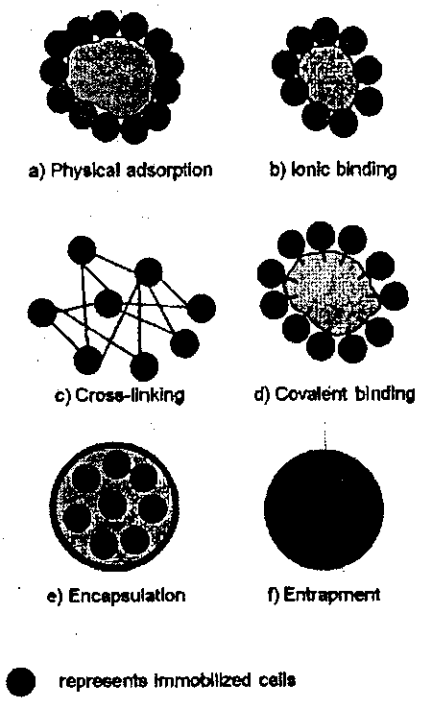

Fig. 7.1 Cell immobilization techniques [65], Reprinted by permission of the publisher

encapsulation technique wraps the cells inside a shell (matrix) as shown in Fig. 7.1e. As a result, the two techniques provide more protection to the cells and are sometimes grouped together as a single category of cell immobilization. There are a number of successful applications of entrapped and encapsulated cells in environmental, pharmaceutical, and food industries. Entrapment matrices are known to be more durable than encapsulation matrices and therefore are more suitable for field applications.

7.2.1 General Principles of Cell Entrapment

Cell entrapment procedures normally consist of two steps: (1) mixing of cells and viscous-liquored matrix and (2) gelation (Fig. 7.2) [38]. The mixing of cells and matrix is performed by dispersing the cells in the matrix, which can be accomplished by simple blending techniques such as magnetic stirring and propeller mixing. There are two common approaches for gelation: droplet and plated gelations. In droplet gelation, the mixture of cells and matrix is dropped into a gel formation solution to produce spherical beads using a syringe or a peristaltic pump (Fig. 7.2). In plated gelation, the mixture of cells and matrix is poured into a tray containing a gel formation solution and the formed gel is cut into small cubes (Fig. 7.2).

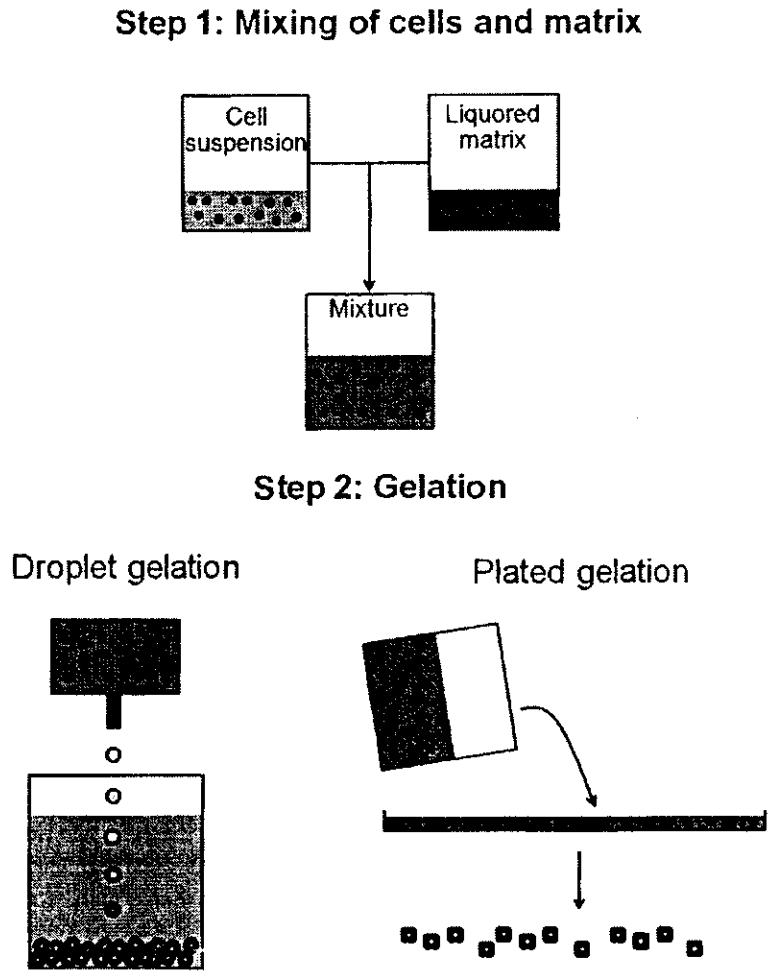

Fig. 7.2 General cell entrapment procedures

The gelation takes place via several processes including ionictropic gelation, temperature-induced gelation, organic polymerization, and phase separation depending on entrapment matrices [39]. Ionictropic gelation is a cross-linking between a matrix (polyionic polymer) and a cation in the gel formation solution. For example, calcium alginate is formed through ionictropic gelation. Temperature-induced gelation is a phase transition at different temperatures. The examples of the temperature-induced gelation are agarose and gelatin formations. Organic polymerization occurs through a reaction between monomers. Common cell entrapment matrices such as polyacrylamide, polymethacrylate, and PVA are the products of organic polymerization. During polymerization, a cross-linking agent may be added for a better gel network. The last process is a phase separation which the cells are

extracted by a gel formable solvent. The phase separation process is quite limited in use since the gel formable solvent could severely damage the viability of the cells.

7.2.2 Widely Used Cell Entrapment Matrices and Procedures

For environmental applications, cell entrapment matrices can be categorized into two types: natural and synthetic. Natural matrices, such as CA, CN, agarose, and gelatin, are polysaccharides produced from algae or seaweed while synthetic matrices are man-made polymers, such as PVA, CTA, polyethylene glycol, and polyacrylamide. The criteria for matrix selection are summarized in Table 7.1. The principles, descriptions, and cell entrapment and de-entrapment procedures for only selected matrices including CA, CN, PVA, and CTA are reviewed below. Note that the de-entrapment process is needed for evaluating the cell number and growth inside the matrix.

Table 7.1 Criteria for cell entrapment matrix selection

Property	Criterion	Reference
Surface area	Large	Kourkotus et al. [65]
Handling and regeneration	Easy	Kourkotus et al. [65]
Cell retention	High	Kourkotus et al. [65]
Cell viability	High	Kourkotus et al. [65]
Biological activity	High	Jen et al. [35] Kourkotus et al. [65]
Porosity/Diffusivity	High	Jen et al. [35] Kourkotus et al. [65] Leenen et al. [79]
Mechanical and chemical stability	High	Kourkotus et al. [65] Leenen et al. [79]
Preparation procedure	Easy	Kourkotus et al. [65] Leenen et al. [79]
Solubility	Low	Leenen et al. [79]
Biodegradability	Low	Leenen et al. [79]
Cell growth	Possible	Leenen et al. [79]
Cost	Low	Leenen et al. [79]

7.2.2.1 Calcium Alginate

Calcium Alginate Chemistry and Gelation

Alginate is one of the pioneer materials used for cell entrapment. It is a non-toxic natural polysaccharide extracted from brown algae, seaweed and bacteria such as *Laminaia hyperborean*, *Macrocystis pyrifera*, *Ascophyllum nodosum*, and *Azotobacter vinelandii* [40–42]. It is a chain of 1–4 linked β-D-mannuronate (M) and α-L-guluronate (G) in different compositions, sizes, and patterns depending

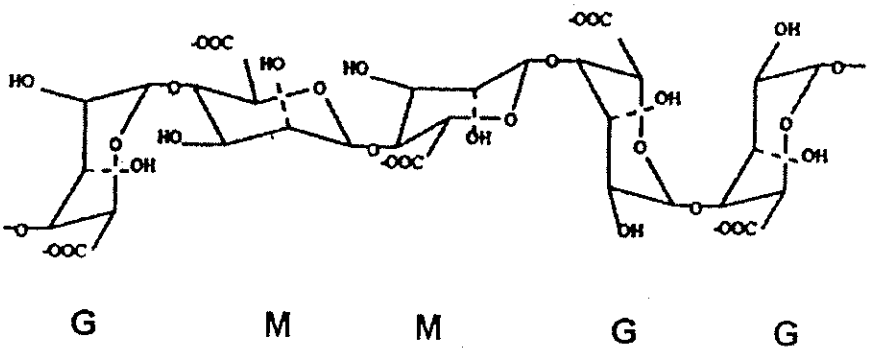

Fig. 7.3 Chemical structure of alginate [42]

on the sources (Fig. 7.3). Alginate is commercially available as a sodium salt of alginate.

Gelation of alginate is a cross-linking of alginate with divalent cations, such as Ca^{2+}, Ba^{2+}, and Sr^{2+}. Calcium is the most widely used cross-linker and the CA entrapment is simple, quick, and inexpensive. Normally, CA gel is prepared by the droplet gelation method. When the sodium alginate solution is in contact with the Ca^{2+} solution, a semi-solid structure is formed immediately in its outer layer. The Ca^{2+}solution then passes through the outer layer to form the gel structure for the entire alginate bead. The chemical structure of alginate affects its properties, stability, and biodegradability [42]. Alginate containing high G content, especially with a long GG structure, provides high gel strength and low shrinkage. This is because the GG block favors more cation bindings, which consequently lead to higher gel stability.

The CA gel is stable in broad ranges of pH (pH of 3–10) and temperature (up to 85°C) [41]. The drawbacks of the CA gel are gel abrasion and swelling under some conditions [4, 40, 41, 43]. The CA gel beads are demolished in the environment containing high concentrations of divalent cations (except Ca^{2+}), phosphate, and chelating agents and swell in the presence of monovalent cations.

Procedures of Calcium Alginate Cell Entrapment and De-Entrapment

The CA cell entrapment procedures are similar in most previous studies. The following procedure is one of the successful methods which was used in several environmental applications [20, 44, 45]. Sodium alginate powder is dissolved in deionized water (DI) at 2% (w/v). To prevent agglomeration, the powder is slowly added into stirred DI. The solution is stirred until all the powder is totally dissolved, which could take up to 12 h. A liquid medium containing microbial cells is centrifuged at 7000 rpm for 10 min to obtain concentrated cells, which are then uniformly mixed with the sodium alginate solution. The mixture is dropped into a calcium chloride solution of 3.5% (w/v) using a peristaltic pump at a flow rate of 3 mL/min (bead size of 2.0–5.0 mm depending on the pump head). The droplets

remain in the solution for 2.5–3.0 h for the formation and hardening of spherical beads.

As mentioned earlier about the variation of the CA entrapment procedure, the chemical concentrations, centrifugation speed, dropping rate of the cell-alginate mixture, and hardening time may be modified for enhancing the bead durability and/or convenience of bead preparation. For instance, the hardening time of the CA gel beads normally ranges from 0.5 to 3.0 h; however, it has been shown that more durable beads can be obtained by increasing the hardening times to overnight [20]. The CA entrapped cells can be de-entrapped hy vigorous vertical shaking in a 0.3 M sodium citrate solution at pH 5 [20, 46] or 50 mM phosphate buffer at pH 7 [40].

7.2.2.2 Carrageenan

Carrageenan Chemistry and Gelation

Carrageenan is another common matrix for cell entrapment. It is produced from red seaweed [38, 43, 47]. Its structure contains 1,3-linked β-D-galactose and 1,4-linked 3,6-anhydro-α-D-galactose. There are three types of CN based on the number and position of sulfonation: kappa (κ), lambda (λ), and iota (ι) (Fig. 7.4). Lambda-CN is water soluble; therefore, it is not suitable for cell entrapment. Between κ and ι-CN, κ-CN is a better cell entrapment matrix since it has a stronger gel network.

The CN gelation process can be either ionictropic or temperature-induced (cooling) gelations. Similar to CA gel, κ-CN gel can be formed with different ions, such

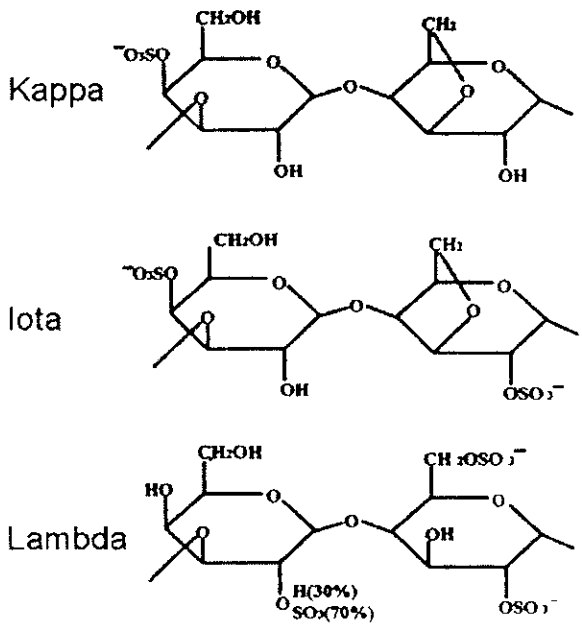

Fig. 7.4 Chemical structure of carrageenan [47]

as K^+, NH_4^+, Ca^{2+}, Cu^{2+}, Mg^{2+}, Fe^{3+}, and amines [38, 43, 47]. Additionally, the strength of κ-CN gel can be improved by adding polymers such as galactomannans, locust bean gum, and taragum [38, 43]. Normally, κ-CN entrapped cells have relatively high biological activities and in turn broad utilizations. However, the applications at high temperatures are not suitable because κ-CN gel dissolves [48].

Procedures of κ-Carrageenan Cell Entrapment and De-Entrapment

The κ-CN cell entrapment can be accomplished by both droplet and plated gelations. The droplet method is suitable for a laboratory scale since the setup is simple. However, the droplet method would be time-consuming for preparing a large volume of entrapped cells; the plated method is more appropriate for it. The following procedure is a general droplet method implemented in several studies [20, 47, 49]. Kappa-CN powder is dissolved in stirred DI at a temperature of 50°C and the solution is allowed to cool down to 35°C. Then, concentrated microorganisms are mixed with the κ-CN solution. The mixture is dropped into 0.3 M potassium chloride and 0.18 M calcium chloride solutions for gel formation and gel hardening, respectively. The final κ-CN concentration is about 2–5% (w/v). The de-entrapment procedure for the κ-CN entrapped cells involves continuous shaking in a 1% sodium citrate solution at 37°C [20, 27].

7.2.2.3 Polyvinyl Alcohol

Polyvinyl Alcohol Chemistry and Gelation

PVA is a polymer that can be prepared in the forms of film and hydrogel with high mechanical strength and durability [4, 21, 50]. Similar to CA and CN, PVA is non-toxic even though it is a synthetic polymer. Therefore, it does not negatively affect both microorganisms and environment. Raw PVA appears in a white and free-flowing granule. The chemical structure of PVA is shown in Fig. 7.5. The properties of PVA are based on the polymer chain length (molecular weight) and degree of hydrolysis. Polyvinyl alcohol with high molecular weights and degrees of hydrolysis has high mechanical stability and low water solubility [51].

Several gelation techniques are available for producing PVA gels for cell entrapment including boric acid-PVA (BPVA), freezing and thawing of PVA (FPVA), and phosphorylated-PVA (PPVA) methods. The BPVA technique is the simplest and most economical. The technique is a one-step droplet gelation method [52]. The BPVA gelation process is a cross-linking of boron and PVA as shown in Fig. 7.6. The BPVA hydrogel beads present high mechanical strength and durability. However,

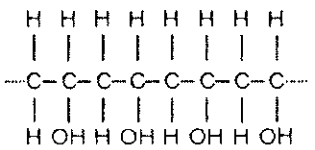

Fig. 7.5 Chemical structure of PVA

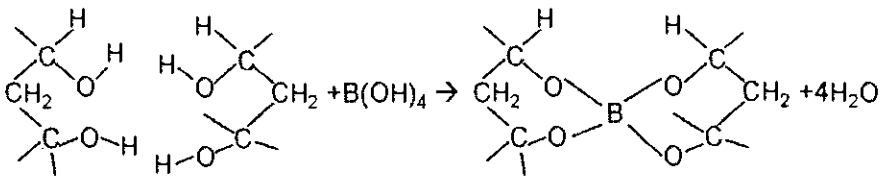

Fig. 7.6 Reaction of PVA-boric gelation

there are two potential problems associated with the technique: cell damage in the boric acid solution and PVA bead agglomeration [21, 53]. Several researchers modified the procedure to solve these problems, such as additions of calcium alginate and activated carbon [53, 54].

The FPVA technique is based on physical cross-linking during temperature-induced condition. Under cryotropic conditions, hydrogen bonds between OH groups of the PVA polymer chain(s) occur either within the chain (intramolecular) or between two chains (intermolecular) [55]. Although this technique provides a strong PVA cryogel, the freezing condition could affect cell viability.

Chen and Lin [21] developed a PPVA method that reduces the boric acid contact time and consequently cell damage associated with the boric acid-PVA method. This modified technique not only decreases the cell damage by boric acid but also increases the strength and durability of entrapped cell beads. The PPVA technique is a two-step droplet gelation method including spherical bead formation and hardening. In the first step, spherical bead formation, the PVA-boron cross-linking occurs according to the reaction shown in Fig. 7.6. In the second step, bead hardening, spherical beads are left in a sodium phosphate solution to increase the surface gel strength through PVA phosphorylation (Fig. 7.7) [56].

Procedures of Phosphorylated-Polyvinyl Alcohol Cell Entrapment and De-entrapment

As mentioned above that BPVA and FPVA entrapment protocols may affect cell viability, therefore, only PPVA cell entrapment is reviewed here. The following PPVA cell entrapment procedure is according to Siripattanakul et al. [57]. The procedure was modified from Chen and Lin [21] for preventing PVA bead agglomeration during the PVA-boron cross-linking step. The modified cell entrapment procedure begins with dissolving PVA in stirred DI at temperature of 60–80°C and letting the solution cool down to room temperature. Microbial cells are centrifuged at $4000 \times g$ for 10 min and then mixed with the PVA solution. The mixture is dropped into a saturated boric acid solution in a 1-l cylinder and remains in the solution for 30–45 min

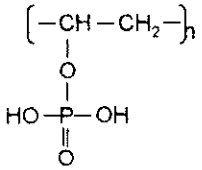

Fig. 7.7 Structure of PVA phosphorylation [56, p. 654], Copyright (2004 and John Wiley & Sons, Inc.): Reprinted with permission of John Wiley & Sons, Inc

to form spherical beads. Note that in the method by Chen and Lin [21], the cell-PVA mixture is dropped into a stirred boric acid solution for 10–120 min. Then, the formed hydrogel beads are then soaked in a 1.0 M sodium phosphate solution at pH 7 for 60 min for hardening. The final PVA concentration is 10% (w/v).

The de-entrapment procedure of the PPVA involves adding the PPVA entrapped cells into DI and heating to about 60°C. As mentioned above, the de-entrapment process is normally performed for measuring cell number and/or growth inside the matrix. This thermal de-entrapment may damage the cell viability making plate counting of the de-entrapment product an unsuitable method for quantifying the cells in the PPVA matrix. Measuring the cell mass (represented by volatile suspended solids) after the de-entrapment is an alternative to this limitation [20].

7.2.2.4 Cellulose Triacetate

Cellulose Triacetate Chemistry and Gelation

Cellulose is a natural polymer extracted from plants. Its structure is a chain of organic compounds containing glucose molecules of which the number and location in the chain vary based on the sources [43]. Natural cellulose itself is not appropriate for cell entrapment; however, modified cellulose compounds via chemical processes, such as esterification and etherification, can form fiber networks. Examples of the modified cellulose compounds are cellulose azide, diazo cellulose, and CTA. Cellulose triacetate has been applied as a cell entrapment matrix since 1980s. The CTA entrapped cells were first developed for food technology applications. The CTA cell entrapment matrix is rubber-like, which is different from the other entrapment media described earlier. The CTA entrapped cells have very high mechanical strength. It was reported that the CTA entrapped cells can be used continuously for more than 8 years [13, 14].

Procedure of Cellulose Triacetate Cell Entrapment

The CTA entrapped cells are prepared by the plated gelation. A procedure to prepare CTA entrapped cells was introduced by Kolarik et al. [58]. Later, Yang and See [59] modified it to ease the preparation. The modified procedure has been used in several studies [6, 12–14, 34]. Cellulose triacetate powder is dissolved in methylene chloride at a concentration of 10% (w/v). Concentrated microbial cells are uniformly mixed with the CTA solution. Then, the mixture is plated into toluene for hardening. The hardened CTA sheet is cut to small cubes and washed with water to rinse the residual chemicals. Currently, there is no procedure for CTA de-entrapment.

7.2.3 Advantages and Drawbacks of Entrapped Cells

There are several advantages of entrapped cells over suspended cells [60, 61]. Basically, cell entrapment leads to the enhancement of both biological and mechanical stabilities. The entrapment matrix can alleviate physicochemical challenges, such as temperature, pH, solvents, shear, and heavy metals. Other advantages of entrapped cells include high biomass concentration, no need for cell separation,

increased product yield and stability, increased reaction selectivity, and versatility in the selection of the reactor. Several studies reported that entrapped cells provided better waste treatment performances and/or are more durable than free cells [7, 20, 62–64]. For example, in a previous study, PPVA entrapped cells were used for removing total organic carbon compared to free cells. The results indicated that the PPVA entrapped cells had substantially higher specific growth and substrate utilization rates [20]. The main drawbacks of entrapped cells are metabolic changes, cell morphology changes, substrate and chemical growth factor diffusion limitations, and inconsistent growth pattern [61, 64].

7.3 Applications of Entrapped Cell Bioaugmentation

In the past two decades, bioaugmentation and cell entrapment processes have been separately applied in the environmental field. Examples of bioaugmentation applications include removal of 2,4-dichlorophenoxyacetic acid, 3-chlorobenzoate, 3-chloroaniline, diesel (oil spills) [2, 3, 64] whereas the cell entrapment has been applied for removing phenol, dyes, and cyanide [1, 28–32]. Although both processes alleviate several problems associated with traditional contaminant removal schemes, the roles of the two processes are different. Bioaugmentation provides a number of specific or acclimated contaminant-degrading cultures whereas cell entrapment maintains the cultures in the system and protects them from stresses.

Combining bioaugmentation and cell entrapment results in a novel process, called entrapped cell bioaugmentation, which inherits the benefits of both processes. Entrapped cell bioaugementation has been studied for environmental applications only in recent years. The applications involved the degradation of collective and specific pollutants in wastewater treatment plants and contaminated sites. Although entrapped cell bioaugmentation has not been applied at field scales since it is relatively new, the bench-scale results thus far are very promising. The technique can retain effective contaminant-degrading cultures within the target systems and the matrices can protect the cells from environmental stresses. Table 7.2 presents a summary of previous studies on entrapped cell bioaugmentation for environmental applications. Since the technology has been studied mainly for wastewater treatment and site remediation, only these two categories of applications are reviewed below for each cell entrapment matrix separately. For matrices that have not been used for entrapped cell bioaugmentation, their technological outlook is provided.

7.3.1 Wastewater Treatment

7.3.1.1 Calcium Alginate Entrapped Cell Bioaugmentation

Calcium alginate is the most common matrix studied in the applications of entrapped cell bioaugmentation for wastewater treatment. There were several successful applications of the CA entrapped cell bioaugmentation for removing toxic compounds in domestic and industrial wastewater such as oil, phenol, and cresol, as listed in Table 7.2. The bioaugmented cultures were pure or enriched mixed cultures.

Table 7.2 Summary of studies on entrapped cell bioaugmentation for contaminant removal

Entrapment matrix	Microorganism	Contaminant	Environmental medium	Reference
CN	*Pseudomonas* sp. UG30	Pentachlorophenol	Water	Cassady et al. [76]
CN	*Pseudomonas* sp. UG30	Pentachlorophenol	Soil	Cassady et al. [77]
CA	Enriched mixed cultures	Phenol and cresol	Wastewater	Guiot et al. [66]
CA	Enriched mixed cultures	Phenol and cresol	Wastewater	Hajji et al. [78]
FPVA	Enriched microorganisms	Diesel	Soil	Cunningham et al. [64]
BPVA modified by sodium alginate or activated carbon	*Zoogloea* sp.	Phenanthrene and pyrene	Soil	Li et al. [54]
CA and agar	*Rhodobacter shaeroide* S *Rhodobacter shaeroide* NR-3	Cooking oil	Synthetic wastewater	Takeno et al. [68]
CA	*Rhodococcus erythropolis* NI86/21	Atrazine	Water and soil	Vancov et al. [33]
PVA	Denitrifying sludge	Nitrate	Agricultural drainage	Hunt et al. [74]
CA	Mixed culture	Nitrate	Synthetic agricultural infiltrate	Siripattanakul et al. [45]
PPVA	Acclimated mixed culture and *Agrobacterium radiobacter* J14a	Atrazine	Synthetic wastewater	Siripattanakul et al. [57]
CA	*Sphingomonas chlorophenolica* PCP-1	Pentachlorophenol	Groundwater	Yang and Lee [24]
CA	Recombinant *Escherichia coli*	Coumaphos, chlorferon, and diethylthiophosphate	Waste cattle dip solution	Ha et al. [18]

Table 7.2 (continued)

Entrapment matrix	Microorganism	Contaminant	Environmental medium	Reference
PPVA	*Agrobacterium radiobacter* J14a	Atrazine	Synthetic agricultural infiltrate	Siripattanakul et al. [7]
PPVA	Mixed culture	Atrazine	Synthetic agricultural infiltrate	Siripattanakul et al. [75]

The calcium alginate matrix contains numerous pores while providing a strong network for cell restriction and proliferation as shown in scanning electron microscopic (SEM) images in Fig. 7.8. Large numbers of cells are present both inside and on the surface of the matrix. It has been reported that CA entrapped cell bioaugmentation greatly improves the wastewater treatment operation and efficiencies. For example, Guiot et al. [66] examined phenol and cresol removal by the bioaugmented CA entrapped acclimated mixed cultures in an upflow anaerobic sludge blanket reactor (UASB). The results showed that the UASB with the bioaugmented CA

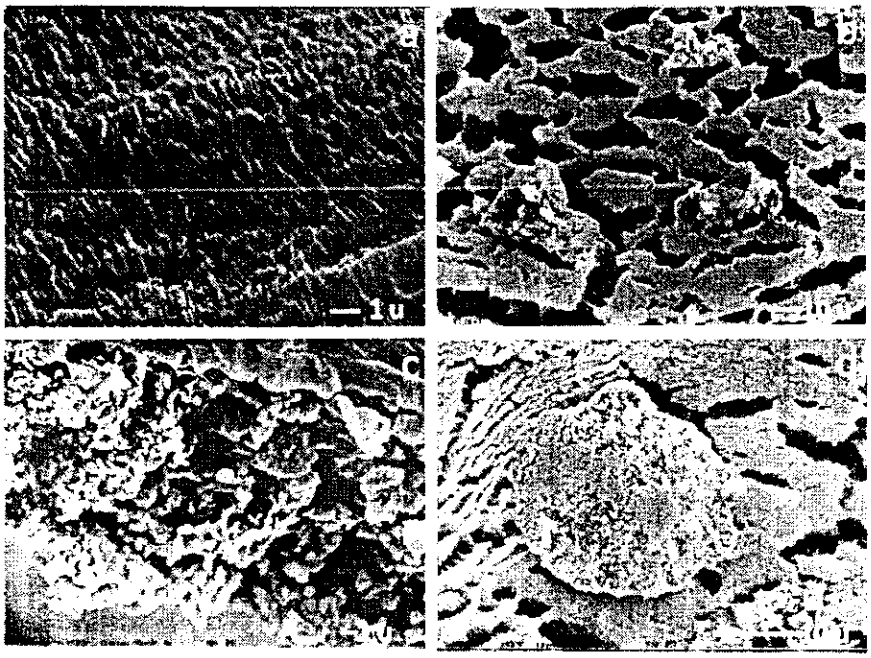

Fig. 7.8 SEM images of calcium alginate entrapped nitrifying bacteria: (**a**) Bead surface after entrapment, (**b**) Interior bacterial floc after entrapment, (**c**) Surface rupturing bacterial colony after experiments, and (**d**) Interior bacterial colony after experiments [44]

entrapped cells could remove the contaminants much better than the UASB alone. The maximum specific phenol, *p*-cresol, and *o*-cresol treatment activities of the system with the entrapped cells were approximately 13, 16, and 8 times higher than those without the entrapped cells. Additionally, the bioaugmented system reached a steady state much quicker and provided higher process stability.

Although CA is a popular entrapment material, there have been reports on its susceptibility to degradation [4, 67]. However, some studies found that the CA entrapped cells are durable and perform well after several rounds of reutilization. A good example is a study by Moutaouakkil et al. [30] which reported that the CA entrapped cells removed toxic azo dye at high concentrations efficiently even after reutilizing them 7 times. Lately, the modified or amended CA was developed for improving contaminant removal efficiencies and the matrix stability. Activated carbon, bentonite, and skim milk are among the amendments [17].

7.3.1.2 Carrageenan Entrapped Cell Bioaugmentation

The utilization of CN entrapped cells is relatively limited among the selected matrices. To date, there has been no CN entrapped cell bioaugmentation application for wastewater treatment. There are only a few basic studies on the uses of the CN entrapped cell inoculation as a treatment scheme by itself. For example, κ-CN entrapped cells were applied to remove total organic carbon [20] and glucose [49] in liquid systems.

The limitation on the CN entrapped cell applications may be attributed to the weakness of the material. The traditional κ-CN entrapped cell preparation requires warm temperatures (35–55°C) for dissolving the κ-CN powder, which could damage the viability of cells. Additionally, the κ-CN entrapped matrix is sensitive to cations, such as K^+ and NH_4^+ leading to easy gel abrasion. However, the κ-CN structure is appropriate for cell proliferation. Godia et al. [69] reported a large number of proliferated cells in κ-CN after 1-day fermentation (Fig. 7.9). The modified κ-CN matrices by clay or skim milk amendments were developed for better gel strength [70]. Through advancements in material science and technology, it is possible that

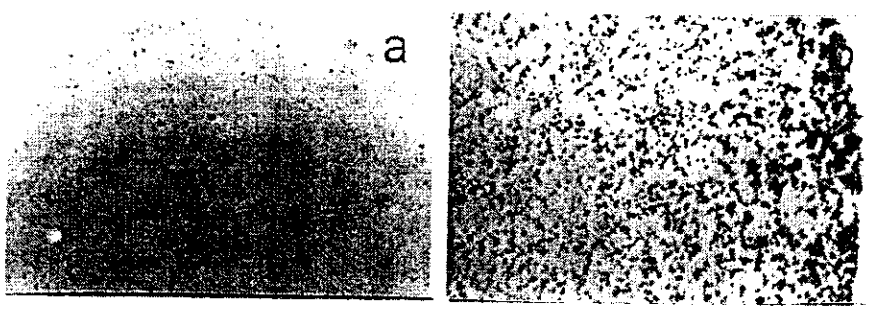

Fig. 7.9 Images of cell distribution in carrageenan matrix: (**a**) After entrapment and (**b**) After 1-day fermentation [69]

the strength of κ-CN will be improved in the near future and more applications of κ-CN entrapped cells including bioaugmentation will then take place.

7.3.1.3 Polyvinyl Alcohol Entrapped Cell Bioaugmentation

Up to date, there has been no work on PVA entrapped cell bioaugmentation for wastewater treatment. Based on several previous successful cases of PVA entrapped cell applications as a stand alone wastewater treatment process, PVA entrapped cell bioaugmentation will likely be attempted soon. The PVA entrapped cells successfully removed more than 90% of contaminants such as organic carbon, nitrogen, 2-methylnaphthalene, and phenol [21, 28, 71–73]. Sharanagouda and Karegoudar [72] reported that 2-methylnaphthalene removal efficiencies by PVA entrapped cells (60 to > 90%) are higher than those by corresponding free cells (20–60%).

The PVA matrices provide a proper microstructure for the contaminant-degrading cultures [57]. Figure 7.10 presents the SEM images of PPVA entrapped atrazine degraders, which reveals two porous bead layers. The outer layer has less porosity providing an effective structure for cell retention. Additionally, PVA matrices were proven to be a good entrapment material in terms of mechanical, chemical, and biological stabilities. The matrices were found unbroken after 6-month utilization [28], reusable more than 30 times without losing degradation ability for

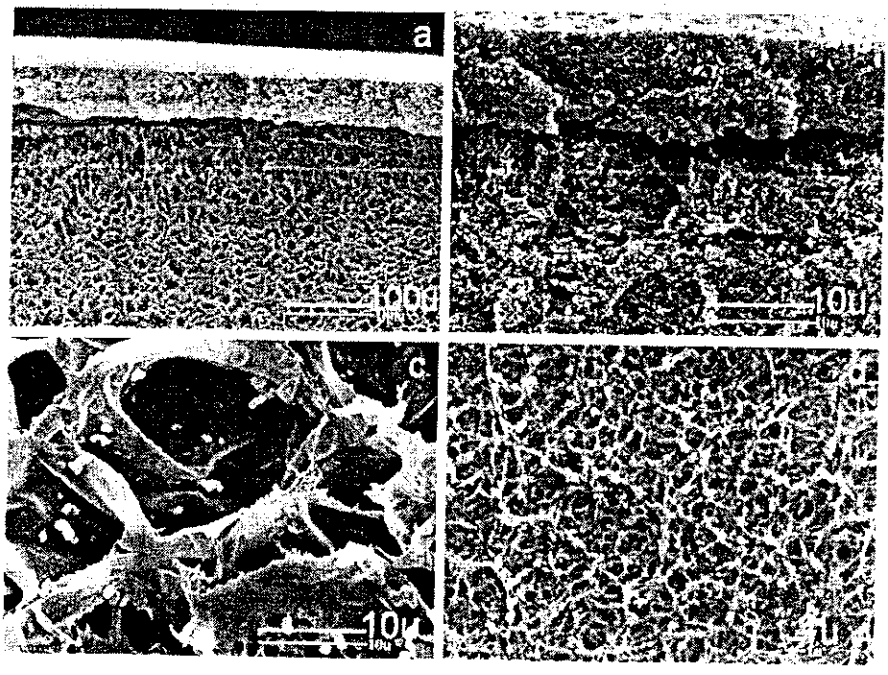

Fig. 7.10 SEM images of PPVA entrapped cells: (**a**) Cross-section at 250×, (**b**) Exterior layer at 3000×, (**c**) Interior layer at 3000×, and (**d**) External surface at 5000× [57]

2-methylnaphthalene [72], and reusable more than 50 times at various pH and temperatures [73]. The PVA entrapment process only slightly affects the cell viability based on the viable plate count and a fluorescence based assay [28].

7.3.1.4 Cellulose Triacetate Entrapped Cell Bioaugmentation

There have been less numbers of applications of CTA entrapped cells for wastewater treatment compared to CA and PVA. This could be because the CTA entrapped cell preparation procedure involves the use of toxic chemicals as mentioned above. The chemicals may severely damage the contaminant-degrading cultures and pose health risk to the personnel involved. In addition, hazardous wastes are generated from the procedure. Advantages of the CTA matrix include very high mechanical and chemical strengths. The entrapped cells can be used for longer than eight years without the breakage of the matrix. Even though the entrapment procedure could be very harmful to microorganisms, high contaminant removal efficiencies by CTA entrapped cells have been reported [6, 12–14, 34, 59].

Cellulose triacetate entrapped cell bioaugmentation is utilized in a novel wastewater process called immobilized cell augmented activated sludge (ICAAS), which was developed to improve the ability of activated sludge process to degrade contaminants (Fig. 7.11). The ICAAS system is an activated sludge system with an off-line enricher reactor growing CTA entrapped cells, which are induced to have specific activities such as toxic contaminant degradation, nitrification, and denitrification [6, 34]. The enriched entrapped cells are used for bioaugmentation in the aeration tank. Once they are less active due to unfavorable conditions in the aeration tank such as the absence of the target contaminants and/or competition with indigenous microorganisms, they are returned for reactivation in the enricher reactor and in the mean time replaced by the active cells from the enricher reactor.

Jittawattanarat et al. [6] investigated pentachlorophenol (PCP) removal by completely mixed activated sludge (CMAS) and ICAAS processes (Fig. 7.12). Synthetic wastewater containing PCP at 40 mg/L was used. The ICAAS systems with and without powder activated carbon (PAC) entrapped along with the cells removed PCP more than the CMAS system (no bioaugmentation) as shown in Fig. 7.12.

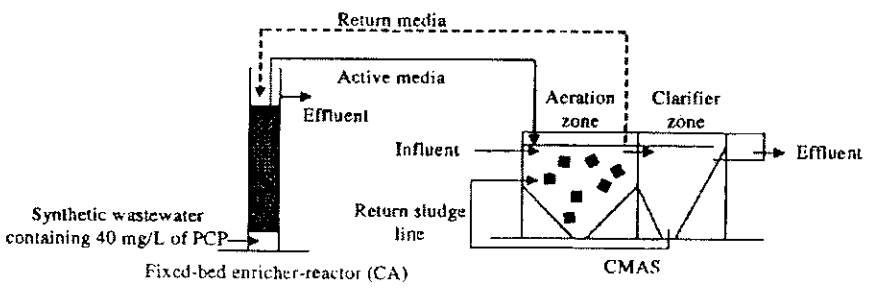

Fig. 7.11 A diagram of immobilized cell augmented activated sludge system [6]. Reprinted by permission of the publisher

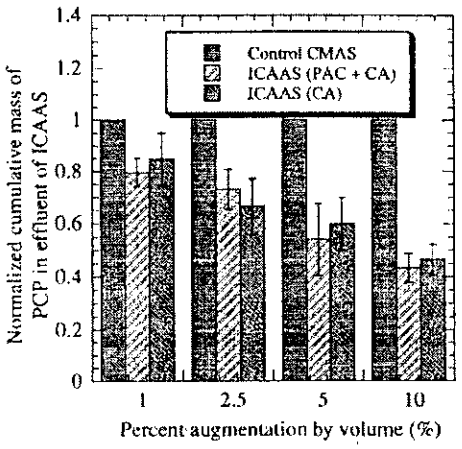

Fig. 7.12 Normalized pentachlorophenol concentrations in effluent from CMAS and ICAAS systems at different bulk volumetric bioaugmentation ratios [6]. Reprinted by permission of the publisher

At 10% bioaugmentation by volume, the cumulative mass of PCP in the effluent of ICAAS was about 50% less than that of CMAS. The results further indicated that PCP biodegradation and adsorption took place in the ICAAS systems but biodegradation by the bioaugemented entrapped cells was the main removal mechanism.

7.3.2 Site Remediation

7.3.2.1 Calcium Alginate Entrapped Cell Bioaugmentation

There have been four studies on CA entrapped cell bioaugmentation for site remediation. The target contaminants were atrazine, nitrate, PCP, coumaphos, chlorferon, and diethylthiophosphate (Table 7.2). Calcium alginate entrapped cells were bioaugmented for removing contaminants in soil, groundwater and infiltrate. Stripattanakul et al. [45] studied the use of CA entrapped cell bioaugmentation for denitrifying synthetic agricultural infiltrate using a laboratory sand column setup at 20 ± 2°C. The CA entrapped cells achieved nitrate removal of more than 90% within 8 hr compared to about 50% by corresponding free denitrifiers.

7.3.2.2 Polyvinyl Alcohol Entrapped Cell Bioaugmentation

Only a few studies dealing with the uses of PVA entrapped cell bioaugmentation for site remediation have been reported. As presented in Table 7.2, PVA entrapped cell bioaugmtentation was used for phenanthrene, pyrene, and atrazine treatment in soil and infiltrate [7, 54, 75]. Phosphorylated polyvinyl alcohol entrapped cell bioaugmentation is a potential method for site remediation since the matrix is durable and has no negative effects on microorganisms and environment [4, 21, 57].

Siripattanakul et al. [7] introduced a PPVA entrapped cell bioaugmentation scheme for removing atrazine in agricultural infiltrate and in turn protecting groundwater quality. In their laboratory-scale sand column study, *Agrobacterium*

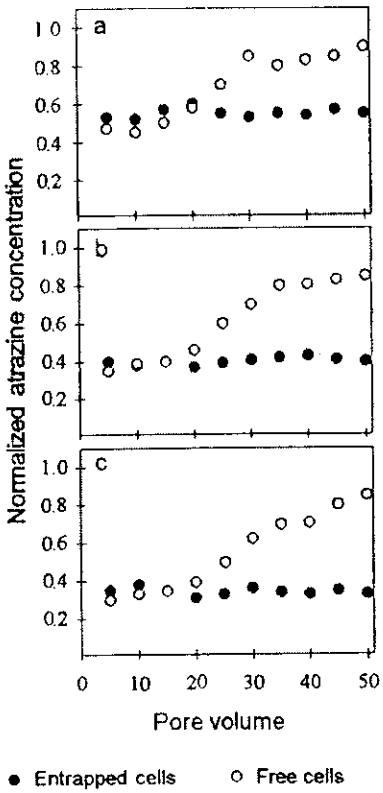

Fig. 7.13 Long-term column experimental results of cell bioaugmentation for treating atrazine in infiltrate using PPVA entrapped and free cells at cell loadings of (**a**) 300, (**b**) 600, and (**c**) 900 mg dry cells/L empty bed volume [7], Reprinted by permission of the publisher

radiobacter J14a (J14a), a known atrazine degrader, entrapped in PPVA was applied on a top sand layer and its ability to treat atrazine in a synthetic infiltrate was compared to bioaugmented free J14a cells. For a short term experiment (6 pore volumes), the atrazine removal efficiencies of up to 99% were achieved for both the free and entrapped cells. However, for a long-term experiment (50 pore volumes), the entrapped cell system provided consistent atrazine removal efficiency while the atrazine removal by the free cells declined gradually because of the cell loss (Fig. 7.13).

7.3.2.3 Carragenan and Cellulose Triacetate Entrapped Cell Bioaugmentation

Two investigations on the use of κ-CN entrapped cell bioaugmentation for site remediation have been conducted [76, 77]. Pentachlorophenol was the contaminant in both studies which focused on different environmental media, soil and water. Entrapped cells removed PCP from synthetic wastewater 3-times higher than free cells [76]. For the soil study, PCP removal using free cells, entrapped cells, CN matrices without cells, and sterile soil (no cells) was compared [77]. The results indicated that the bioaugmented entrapped cells performed more efficiently reducing PCP concentration about 64% while PCP removal in the systems with free cells,

CN matrices, and sterile soil systems ranged 16–18%. However, based on the CN chemistry, the κ-CN entrapped matrix is sensitive to cations, the application for site remediation may not be practical because environmental media (soil, infiltrate, and groundwater) normally contain several types of ions.

There has been no work on CTA entrapped cell applications for site remediation. The CTA matrix should be suitable for practical use in site remediation because it is very durable. However, the toxic chemicals used for the CTA entrapped cell preparation may cause additional contamination to the environmental media.

7.4 Conclusions and Future Perspectives

Entrapped cell bioaugmentation is a potential technology for contaminant removal. It provides better cell retention and tolerance compared to traditional planktonic cell bioaugmentation leading to higher contaminant removal efficiencies. In the last decade, studies on the applications of entrapped cell bioaugmentation for wastewater treatment have been mainly on xenobiotic treatment enhancement. The drawbacks associated with entrapped cell bioaugmentation, such as effect of entrapment procedure on cell viability, substrate diffusion limitation, and durability of entrapped cells were discovered and solved. For site remediation, the entrapped cell bioaugmentation investigations have been on remediating runoff, infiltrate, and soil contaminated with urban, industrial, or agricultural residues. Previous work examined the contaminant degradation performance, and augmented cell retention and tolerance under different environmental stresses.

Entrapped cell bioaugmentation is a technology that has been tested at bench scales. The technology has been verified under controlled laboratory conditions for its potential for both wastewater treatment and site remediation applications. Recent laboratory research efforts have been on testing the technology under different environments such as contaminated soils and agricultural infiltration. In the future, entrapped cell bioaugmentation will likely move on to pilot and field scales. Additionally, more environmental applications of the technique may be studied. For example, the technique can be used for in-situ treatment of landfill leachate and degradation of organic solid waste (bioaugmented landfill bioreactors).

The uses of entrapped cells for contaminant removal regardless of the scheme have been in a black-box manner. In-depth investigations on important aspects of entrapped cells including growth, metabolism, morphology, and genetics compared to those of free cells are needed. These understandings at the cellular and molecular levels of entrapped cells would enable more accurate prediction of their behaviors and effective bioaugmentation. Most of the work on entrapped cell bioaugmentation has been limited to laboratory scales. A lack of low-cost and industrial-scale production of entrapped cells is a major impediment for practical applications of the technology. If this issue could be resolved, entrapped cell bioaugmentation, which is a technically capable process, would turn into a sustainable practice and consequently one of the commonly used contaminant removal technologies.

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