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Mini-review: Inducing flocculation of non-floc-forming *Escherichia coli* cells

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Abstract

The present article reviews several approaches for inducing flocculation of *Escherichia coli* cells. The common industrially used bacterium *E. coli* does not naturally have floc-forming ability. However, there are several approaches to induce flocculation of *E. coli* cells. One is induction by flocculants— polyvalent inorganic salts, synthetic polymeric flocculants, or bio-based polymeric materials, including polysaccharide derivatives. Another method is the induction of spontaneous flocculation by changing the phenotypes of *E. coli* cells; several studies have shown that physical treatment or gene modification can endow *E. coli* cells with floc-forming ability. Coculturing *E. coli* with other microbes is another approach to induce *E. coli* flocculation. These approaches have particular advantages and disadvantages, and remain open to clarification of the flocculation mechanisms and improvement of the induction processes.

In this review, several approaches to the induction of *E. coli* flocculation are summarized and discussed. This review will be a useful guide for the future development of methods for the flocculation of non-floc-forming microorganisms.

Introduction

Flocculation is an aggregation phenomenon of microbial cells in which the cells form flocs. The floc-forming capabilities of many microorganisms have been studied (Salehizadeh and Shojaosadati 2001). In the 19th century, flocculation of the yeast *Levure casseeuese* was first reported by Louis Pasteur. Since then, flocculation in 19 other diverse microorganisms, including fungi and bacteria, has been confirmed (Nakamura et al. 1976). In the activated sludge used in wastewater treatment and in pure laboratory cultures, the components of flocs typically include polysaccharides, proteins, and polynucleotides. These bacterial flocs are susceptible to hydrolytic enzymes, such as cellulases, proteases, and deoxyribonucleases (Tago and Aida 1977). Although the complete mechanism for microbial flocculation remains unclear, exopolymeric materials play a key role (Salehizadeh and Shojaosadati 2001). Flocculation can also be applied in industrial fermentation. For example, a smart process has been established for high-performance ethanol production from molasses using flocculating *Saccharomyces cerevisiae* (Morimura et al. 1997). Using flocculating yeast eliminates the costly centrifugation step required for cell recovery during repeated batch ethanol fermentation.

However, not all microbial species can form flocs. For example, the commonly used industrial bacterium *Escherichia coli* does not naturally have floc-forming ability. Therefore, it is necessary to induce the flocculation of such non-floc-forming bacteria. If the objective of flocculation is the removal or inactivation of *E. coli* cells from water, forced flocculation using chemicals or synthetic polymers is effective and inexpensive. If the *E. coli* flocs are to be used as biocatalysts, environmentally friendly or

spontaneous flocculation is desirable. Therefore, it is desirable to have as many choices as possible of methods to flocculate *E. coli* cells for different purposes.

In this review, several approaches to induce flocculation of *E. coli* cells, a typical non-floc-forming microorganism, are summarized and discussed.

Approaches for flocculation of *E. coli* cells

Figure 1 summarizes possible approaches for flocculation of non-floc-forming *E. coli*. These methods can be divided into flocculation of *E. coli* only and flocculation of *E. coli* with other microbes. There are two different approaches for flocculation of *E. coli* only. The first is using flocculants, which is the standard approach to induce the flocculation of non-floc-forming bacteria. Flocculants are categorized into three types—inorganic chemicals, synthetic polymers, and bio-based polymers. The other approach is by changing the phenotypes or properties of the *E. coli* cells to generate floc-forming ability by means of physical treatment or gene modification. Changing the phenotypes or properties is relatively new and is desirable for the biocatalytic application of *E. coli* flocs. If using mixed microbial species, the coculture of *E. coli* cells with other microbes that have a floc-forming ability is a strong tool for inducing flocculation. The details of each approach are described below.

Flocculants

Flocculants are widely used in industrial processes, including wastewater treatment, downstream processing, and food and fermentation processes. As shown in Table 1, the flocculants used can be categorized into three groups; (i) polyvalent inorganic flocculants such as aluminum sulfate; (ii) organic synthetic polymer flocculants, such as

polyethylene imine (PEI) and polyacrylamide derivatives; and (iii) bio-based polymer flocculants, such as chitosan, starch derivatives, and other microbial flocculants (Salehizadeh and Shojaosadati 2001).

Inorganic chemicals

Polyvalent inorganic cations react with water to form hydroxide complexes. These complexes react with phosphorus and suspended particulates to form a relatively insoluble mass, which settles because of many factors, including a reduction of electrical charge. Bacterial cells are colloidal particulates and can be aggregated. In the case of *E. coli* flocculation, polyvalent inorganic cations, such as aluminum nitrate (Rubin and Hanna 1968) and aluminum sulfate (Bulson et al. 1984) have long been known to be suitable flocculants (Table 1). Recently, compounds of rare earth elements, including lanthanum chloride (Zhang et al. 2010) and cerium nitrate (Chen et al. 2010) has been reported to induce flocculation of *E. coli* cells with high efficiency. However, the high cost of rare earth elements is a problem with this approach. Addition of inorganic compounds is an attractive approach to remove *E. coli* cells from raw water because of the easy handling and low cost.

Synthetic polymers

Organic synthetic polymers are the most typical flocculants used because of their high flocculating efficiency and low cost. In particular, cationic polyelectrolytes can drastically change the degree of flocculation at concentration of ppm. PEI is a typical cationic polyelectrolyte and its mechanism of *E. coli* flocculation has been well studied (Treweek and Morgan 1977). Based on adsorption experiments and electrophoretic

mobility and refiltration rate measurements, it was concluded that the primary mechanism of flocculation was not polymer bridging, but adsorption coagulation. Small doses of high molecular-weight PEI species contributed to the formation of a charge mosaic on the oppositely charged *E. coli* cell surface and this resulted in producing rapid flocculation. The adsorbed PEI molecules, not only neutralized the negative surface charge at the adsorption sites, but also caused localized charge reversal because of the presence of excess cationic segments. *E. coli* flocs induced by PEI have been applied as biocatalysts by Zou et al. (Zou et al. 2018). In this study, recombinant *E. coli* expressing *Acidovorax facilis* nitrilase was flocculated with PEI, followed by cross-linking with glutaraldehyde to obtain cross-linked cell aggregates (CLCAs). The CLCAs were investigated as biocatalysts in the regioselective biotransformation of 1-cyanocyclohexaneacetonitile into 1-cyanocyclohexaneacetic acid. The results showed that the half-life of the CLCAs was drastically extended compared with that of free cells.

Flocculation of *E. coli* cells using other synthetic polymers has been summarized by Barany et al. (Barany and Szepesszentgyorgyi 2004). In this work, nonionic and anionic polymers, including polyethylene oxide, polyvinyl alcohol, carboxylchitin, neutral polyacrylamide, hydrolyzed (anionic) polyacrylamide, and polyacrylic acid showed weak flocculation of *E. coli* cells (less than 20% removal). In contrast, flexible cationic polyelectrolytes, such as polydiethylaminoethylmethacrylate (polyDEAEMA) and copolymers of polyDEAEMA with vinylpyrrolidone (polyDEAEMA/VP), acrylamide (polyDEAEMA/AA), and acrylic acid (polyDEAEMA/AC), were excellent flocculants of *E. coli* suspensions; the use of these polymers at concentrations of 15–20 μg/10^9 cells precipitated 90% of *E. coli* cells. On the basis of complex measurements of polymer

adsorption and its effect on the electrokinetic potential and degree of aggregation of cells, it was concluded that the aggregation of *E. coli* cells by polyDEAEMA and copolymers was because of charge neutralization (Barany and Szepesszentgyorgyi 2004).

Bio-based polymers

Traditional flocculants, such as inorganic salts and synthetic polymers, have been proven to provide high flocculation efficiencies in water without large amounts of bacteria. Recently, bio-based flocculants, such as starch, cellulose, chitosan (Salehizadeh and Shojaosadati 2001), and polyglutamic acid (Liu et al. 2018; Liu et al. 2017a) have attracted increasing attention due to their environmentally friendly property, biodegradability, and widespread availability. In particular, developing novel bio-based polymer flocculants with multi-functionality is important. Traditional flocculants, such as those that are inorganic metal-based as well as synthetic organic polymers, have no evident sterilization activities. Moreover, they themselves carry health risks, because of residual metal ions or the release of noxious polymeric monomers into the target water. General bio-based polymer flocculants have been previously reviewed (Salehizadeh and Shojaosadati 2001; Salehizadeh and Yan 2014). Here, we focus on bio-based polymers targeting flocculation of *E. coli* cells with known compositions (i.e., excluding flocculants based on unclarified bio-based polymers or mixtures). Bio-based polymeric flocculants for *E. coli* cells are summarized in Table 1.

Chitosan, the deacetylation product of chitin, appears to be one of the most promising candidates. The unique properties of chitosan mainly arise from the primary amine groups present on the macromolecular backbone. Under acidic conditions, the molecular

chains have a positive charge, suggesting that this biopolymer is quite efficient for flocculating contaminants that have negative surface charges. This fundamental property would clearly provide a benefit in bacterial removal because most bacteria, including *E. coli*, normally carry negative charges on the outside of their cell walls (Agerkvist et al. 1990). Furthermore, there has been unique research into the fractal structures of flocs formed by chitosan in terms of fractal dimensions (Tang et al. 2001), providing a measurement of how the bacteria in the flocs occupy space. Research has also demonstrated that chitosan shows antibacterial activity. To increase the bactericidal effect, quaternary ammonium salt-grafted carboxymethyl chitosan has been developed for *E. coli* flocculation (Yang et al. 2014). This polymer has bactericidal action through the breaking of bacterial cell walls by the grafted quaternary ammonium salts. Chitosan has also been used for the flocculation of *E. coli* cells for biocatalytic applications. Flocculation using chitosan within a wide range of molecular weights and degrees of acetylation can achieve a useful immobilization. On the basis of this technique, *E. coli* cells expressing an omega-transaminase were successfully reused in consecutive batch reactions (Rehn et al. 2013). Despite a very high density of cells in the immobilized preparation, and a fast reaction, diffusion limitation was minimal. Thus, the natural polymer chitosan and its derivatives are highly effective, not only as tools for the removal of bacterial cells from water, but also for the immobilization of bacterial cells for biocatalytic applications.

However, the high cost of chitosan limits its practical applications in water treatment. Starch is an abundant natural resource and much is cheaper than chitosan. Because starch contains large numbers of hydroxyl groups on the saccharide rings, starch can be easily modified chemically for use in various applications by the introduction of

different functional groups onto the backbone. Flocculation of *E. coli* cells has been reported using carboxymethyl-starch-graft-aminomethylated-polyacrylamide (Huang et al. 2016). Under suitable pH conditions, this flocculant both effectively removed turbidity and disrupted *E. coli* cells. In recent work, cationized starch-based flocculants (starch-3-chloro-2-hydroxypropyl triethyl ammonium chloride, St-CTA) containing various quaternary ammonium salt groups on the starch backbone have been used for *E. coli* flocculation (Liu et al. 2017b). St-CTA with a high degree of substitution of CTA improved the removal of contaminants due to the strong cationic nature and the charge naturalization flocculation effect. This flocculant showed better antibacterial effects on *E. coli* cells than on *Staphylococcus aureus* cells, indicating that the thicker cell walls of the Gram-positive bacterium *S. aureus* are harder to break than the walls of *E. coli* cells. In addition, cationized starch-based flocculants substituted with glycidyltrimethylammonium chloride was also reported (El-Naggar et al. 2018). This flocculant has achieved the same flocculation efficiency of aluminum sulphate. As a new bio-based polymer flocculant, lignin nanoparticles (L-NPs) assembled with gelatin was proposed for the *E. coli* flocculation (Yin et al. 2018). Positive charge of gelatin is the driving force for flocculation of L-NPs-gelatin complex.

Change in cellular phenotypes or properties

The addition of flocculants is the most common approach for the flocculation of *E. coli* cells. However, there are other approaches to induce spontaneous flocculation, including by changing the phenotype or properties of *E. coli*. Both physical and biological approaches have been reported (Table 2). In the physical approach, an electric field was applied to enteroaggregative *E. coli* (EAEC) cells. In the biological approach, gene

modification was effective in inducing flocculation of laboratory *E. coli* strains. Both these approaches can maintain a higher survival rate of *E. coli* cells inside the floc structure than that in flocs induced by flocculants. Therefore, spontaneous flocculation induced by a phenotype change may be more suitable for application in fermentation processes than using conventional flocculants.

Physical approaches

EAEC is a diarrheal pathogen defined by a characteristic aggregative adherence to host cells. The EAEC042 strain is known to have important distinguishing properties, such as the positively charged surface protein dispersin and aggregative adherence fimbria (Goochee et al. 1987; Nataro et al. 1985; Sheikh et al. 2002). In normal cases, fimbrial-mediatied EAEC042 adhesion to surfaces leads to biofilm formation. However, Kumar et al. have showed that application of transverse low magnitude alternating current and direct current electric fields in a culture chamber stopped biofilm formation on a glass substrate, and led to flocculation (Kumar et al. 2011). EAEC042 flocs induced by an electric field were more than 200 μm in size with a heterogeneous composition. Both the current and magnitude of the electric field were important parameters for controlling the cell viability in those flocs. These findings show promise for the use of electric fields, not only for the manipulation of bacterial flocs, but also for the treatment of medical instruments in preventing aggregative adherence to surfaces.

Biological approaches

Ojima et al. have demonstrated self-generated flocculation of *E. coli* cells by overexpressing the native *bcsB* gene, which encodes a component of transmembrane

cellulose synthase complexes (Ojima et al. 2015). The resulting flocs had a paper-like structure that was stable. Various *E. coli* laboratory strains including K-12, B, and O formed visible flocs (>1 mm) by overexpressing the *bcsB* gene. The presence of green fluorescent protein (GFP)-expressing *E. coli* cells was confirmed within the floc structure, suggesting that the *E. coli* cells inside the floc structure are likely to be alive. The flocs were sensitive to proteinases, indicating that the main component linking the flocs was proteinous. Both protein analyses and observations of the flocs by transmission electron microscopy indicated the involvement of outer membrane vesicles (OMVs) in the flocculation of *E. coli* cells. OMVs are extracellular vesicles produced by Gram-negative bacteria and are spherical bilayered proteolipids with a diameter of 20–250 nm. OMVs contain outer membrane proteins and lipids, periplasmic proteins, lipopolysaccharides, RNA, and DNA (Lee et al. 2007). Gram-negative bacterial biofilms that have formed either *in vivo* or *in vitro* typically contain numerous OMVs (Mashburn-Warren et al. 2008). It has been observed that the *degP*-deficient mutant (Δ*degP*) cells spontaneously flocculated without overexpression of *bcsB* (Ojima et al. 2015). The *degP* gene encodes a periplasmic protease and its deletion strongly enhances OMV production in *E. coli* cells (Schwechheimer and Kuehn 2013). In contrast, *bcsB*-induced *E. coli* flocculation was greatly suppressed by the deletion of the Δ*dsbA* or Δ*dsbB* gene; these mutants are known to have considerably decreased OMV production (Schwechheimer and Kuehn 2013). These results demonstrate a correlation between the spontaneous flocculation of *E. coli* and enhanced OMV production.

Compared with forced flocculation, self-generated *E. coli* flocs have an advantage for application in fermentation processes, because the cells within the flocs maintain the viability and activity required for use as biocatalysts. For example, ethanol-producing *E.*

coli KO11 cells were endowed with floc-forming ability by overexpression of the *bcsB* gene, without adverse effects on ethanol production (Ojima et al. 2016). In this study, the glucose concentration and culture temperature were important parameters for the flocculation of ethanol-producing *E. coli*. Sedimentation tests showed that the *E. coli* flocs completed sedimentation within 15 min after cessation of shaking, while planktonic cells remained suspended. The advantages of using flocculating *E. coli* KO11 in ethanol production were demonstrated in a repeated batch operation.

When examining the flocculation of *bcsB*-overexpressing *E. coli* cells, mass spectrometry analyses indicated that the elongation factor Ts (Tsf) was dominant protein among the floc proteins (Ojima et al. 2015). Tsf is known to promote the release of guanosine diphosphate by forming an intermediate complex with another elongation factor, Tu, which is involved in the elongation cycle of protein biosynthesis (Zhang et al. 1997). A fusion protein consisting of Tsf and GFP was shown to be expressed throughout the whole floc structure, even in the spaces without *E. coli* cells (Ojima et al. 2018). The amount of Tsf-GFP reached approximately 15% (w/w) of the total floc protein, suggesting that the design and synthesis of a fusion protein with Tsf would enable the display of a recombinant target protein on the structure of an *E. coli* floc. Displaying a recombinant protein on flocs is a promising technique to construct artificial microbial flocs with desired functionalities.

Mixed with other microbes (coculture)

Table 3 shows reported coculture systems of *E. coli* with other microbes for inducing flocculation. Flocculation of *S. cerevisiae* has been much investigated because of its importance in the brewing industry (Soares 2011). Flocculation of *S. cerevisiae* results

from an interaction between a lectin-like protein and mannose residues located on the yeast cell surface. The FLO1 gene, which encodes a cell wall protein, plays an important role in yeast flocculation, which is inhibited by mannose but not by glucose.

Interestingly, Peng et al. found flocculation of *E. coli* cells when they investigated the probiotic effect of yeast cells against diarrhea caused by pathogenic *E. coli* (Peng et al. 1997); *E. coli* cells flocculated in the supernatant of liquid cultures of an antidiarrheal *S. cerevisiae* strain. This flocculation of *E. coli* cells was induced by a glycoprotein released by the yeast cells.

Peng et al. also investigated the coflocculation of *E. coli* cells with a variety of yeast cells (Peng et al. 2001a). The results showed that the *E. coli* strain JM109 strain coflocculated with *Candida utilis* G3, *Dekkera bruxellensis* G1, *Hanseniaspora guilliermondii* H60, *Kloeckera apiculate* K315, *S. cerevisiae* HG, and *Schizosaccharomyces pombe* G21 strains, even though these yeasts are non-floc-forming strains. In addition, the FLO1 deletion mutant of *S. cerevisiae* also coflocculated with *E. coli* cells, suggesting that coflocculation of *E. coli* and yeast cells is independent of any inherent floc-forming-ability of the yeast cells. *S. pombe* showed much less coflocculation than the other yeasts. *S. pombe* is known to have galactose-rich cell walls and the glycosylation mutant *gms1*Δ induced a remarkable amount of coflocculation (Peng et al. 2001b). It was concluded that *E. coli* lectins may have specificity for α-1-6- and α-1-3-linked mannose residues of *S. pombe,* but in wild-type *S. pombe* these mannose residues are shielded by galactose residues.

Coaggregation of *E. coli* with other probiotic strains was also confirmed with *Lactobacillus* spp. (Ekmekci et al. 2009). Coaggregation of *L. acidophilus* S1 with *E. coli* ATCC11229 was observed under both aerobic and anaerobic conditions. The

coaggregation of the strains was greater at acidic pH and decreased after heat treatment. Thus, the coflocculation or coaggregation abilities of probiotic bacteria might enable them to form a barrier that prevents colonization of pathogenic bacteria on host cells.

Potential applications of different flocculation approaches

Table 4 summarizes the advantages and disadvantages of different approaches for inducing *E. coli* flocculation in potential fundamental research and industrial applications. Flocculation of *E. coli* cells using either inorganic chemicals or synthetic polymers is relatively inexpensive and involves easy handling. These flocculants are suitable for wastewater treatment. However, toxicity toward *E. coli* cells and detrimental effects on human health are disadvantages for the application of these flocculants in bioproduction, even though several biocatalytic reactions have been proposed using synthetic polymer-induced *E. coli* flocs. Bio-based polymer flocculants are biodegradable, which gives these flocculants environmentally friendly properties. However, bio-based polymers are not the first choice for wastewater treatment because the cost is relatively high compared with inorganic chemicals or synthetic polymers. A promising application of bio-based polymers is for use in aquaculture feed. Taking advantage of the biodegradability, bio-based polymers can be added to aquaculture ponds to recycle non-utilized proteins and derivative microbial proteins including *E. coli* (Avnimelech 2015). Flocculation of pathogenic *E. coli* cells by an electric field inhibits the biofilm formation and does not need any added flocculants. However, this method cannot be scaled up easily for bulk reactions. The electrical filed method might be applied for the treatment of medical instruments to prevent biofilm infection. Gene modification induces flocculation, without cost or addition of flocculants, because the

growing cells spontaneously form flocs. However, this approach should only be applied in closed reaction systems due to the legal limitations of handling of genetically modified microorganisms. Repeated batch fermentation is one of the promising applications using the sedimentation property of flocs. In addition, the engineering of flocs by further gene modification is also effective in advancing biocatalytic reactions from the laboratory to an industrial scale. Flocculation by coculture with *E. coli* has a complex mechanism, which causes difficulty in the handling of the flocculation. In fundamental research, the coculture approach might provide better understanding of microbial interactions through flocculation. The coculture method is also applicable for use in probiotics because the partner microbes remove the pathogenic *E. coli* cells from the gut in the host animal by the flocculation.

Conclusion

Flocculation of non-floc forming bacteria using added flocculants has been investigated and applied in wastewater treatment. In the case of *E. coli* flocculation, inorganic-metal-based and synthetic organic polymeric flocculants are effective. However, these traditional flocculants have no evident sterilization activities. Moreover, they themselves carry health risks. Therefore, developing bio-based polymer flocculants with multi-functionality is important. Several types of bio-based polymer flocculants with antibacterial properties efficiently remove *E. coli* cells from water, although the cost is still high. In contrast, flocculation by changing the phenotype of *E. coli* is a novel and economical approach. In particular, such biological approaches can maintain a high survival rate of *E. coli* cells inside the floc structure, and thus are suitable for application in fermentation processes. In particular, flocculation induced by gene

modification is a promising technique to construct artificial microbial flocs with desired functions. Coflocculation of *E. coli* and other microbes is a complex phenomenon; coflocculation with probiotic bacteria might enable the formation of a barrier that prevents colonization of pathogenic *E. coli* on host cells.

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Conflict of interest

The authors declare that they have no conflict of interest.

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FIGURE LEGEND

Fig. 1 Categories of approaches for inducing flocculation of non-floc-forming *E. coli* cells.

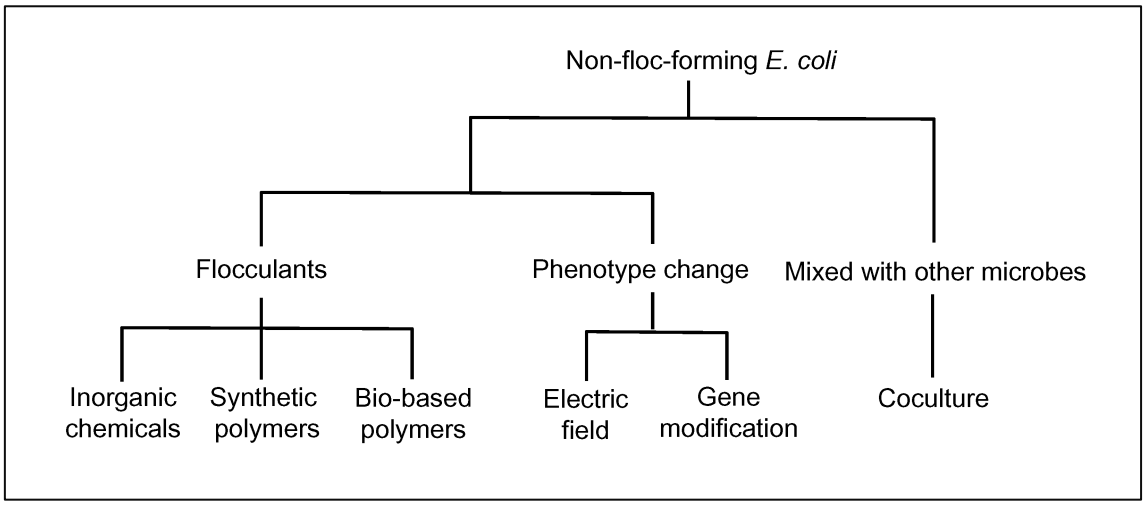

Table 1 Materials for flocculation of *E. coli*

Flocculants	Characteristics	References
Inorganic chemicals		
Aluminum sulfate	Reduction of electrical charge	Bulson et al. 1984
Aluminum nitrate	Reduction of electrical charge	Rubin and Hanna 1968
Lanthanum chloride	Reduction of electrical charge	Zhang et al. 2010
Cerium nitrate	Reduction of electrical charge	Chen et al. 2010
Synthetic polymers		
Polyethyleneimine (PEI)	Positive charge	Treweek and Morgan 1977
Polyethyleneimine (PEI)	Biocatalytic application	Zou et al. 2018
polydiethylaminoethylmetacrylate (polyDEAEMA) and its copolymers	Charge neutralization	Barany and Szepesszentgyorgyi 2004
Bio-based polymers		
Chitosan	Positive charge	Agerkvist et al. 1990
Chitosan	Characterization of fractal structure	Tang et al. 2001
Quaternary ammoniumsalt grafted carboxymethyl chitosan (CMC-g-PDMC)	Increased charge and bridging with bactericide	Yang et al. 2014
Chitosan	Biocatalytic application	Rehn et al. 2013
Carboxymethyl starch-grafted aminomethylated-polyacrylamide (CMS-g-APAM)	Positive charge with bactericide	Huang et al. 2016
Starch-3-chloro-2-hydroxypropyl triethyl ammonium chloride (St-CTA)	Positive charge with bactericide	Liu et al. 2017b
Cationized starch with glycidyltrimethylammonium chloride (GTAC)	Positive charge with bactericide	El-Naggar et al. 2018
Lignin nanoparticles assembled with gelatin	Positive charge	Yin et al. 2018

Table 2 Modification of cellular phenotypes or properties of *E. coli* for flocculation

Approaches	Mechanisms	References
Type1 fimbriae-positive cell at low pH	Fimbriae-to-fimbriae adhesion	Goochee et al. 1987
Electric field	Fimbriae-to-fimbriae adhesion	Kumar et al. 2011
Overexpression of *bcsB* gene	Proteinous component	Ojima et al. 2015
Deletion of *degP* gene	Proteinous component	Ojima et al. 2015

Table 3 Cocultures of *E. coli* with other microbes for flocculation

Strains	Phenomena	References
S. cerevisiae (antidiarrhea strain)	flocculation in the culture supernatant	Peng et al. 1997
C. utilis G3	coflocculation	Peng et al. 2001a
D. bruxellensis G1	coflocculation	Peng et al. 2001a
H. guilliermondii H60	coflocculation	Peng et al. 2001a
K. apiculate K315	coflocculation	Peng et al. 2001a
S. cerevisiae HG	coflocculation	Peng et al. 2001a
S. pombe	coflocculation	Peng et al. 2001b
Lactobacillus spp. (isolated from the lateral vaginal walls)	coaggregation	Ekmekci et al. 2009

Table 4 Advantages and disadvantages of different approaches for inducing *E. coli* flocculation in potential fundamental research and industrial applications

Approaches	Advantages	Disadvantages	Fundamental research	Industrial application
Inorganic chemicals	Low cost/ Easy handling	Toxicity	-	Wastewater treatment
Synthetic polymers	Easy handling	Toxicity	-	Wastewater treatment
Bio-based polymers	Environmentally friendly	High cost	-	Aquaculture feed/ Biocatalytic reactions
Electrical field	Easy handling/ Without flocculants	Small scale	-	Treatment of medical instruments
Gene modification	Low cost/ Without flocculants	Legal limitations of handling	Construction of engineered flocs	Repeated batch fermentation/ Biocatalytic reactions
Cocultures	Low cost/ Without flocculants	Difficult handling	Understanding of microbial interactions	Wastewater treatment/ Probiotics