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One of the principal challenges in the collection of biological samples from air, water, and soil matrices is that the target agents are not stable enough to be transferred from the collection point to the laboratory of choice without experiencing significant degradation and loss of viability. At present, there is no method to transport biological samples over considerable distances safely, efficiently, and cost-effectively without the use of ice or refrigeration. Current techniques of protection and preservation of biological materials have serious drawbacks. Many known techniques of preservation cause structural damages, so that biological materials lose their structural integrity and viability. We review applications of a novel bacterial preservation process, which is nontoxic and water soluble and allows for the storage of samples without refrigeration. The method is capable of protecting the biological sample from the effects of environment for extended periods of time and then allows for the easy release of these collected biological materials from the protective medium without structural or DNA damage. Strategies for sample collection, preservation, and shipment of bacterial, viral samples are described. The water-soluble polymer is used to immobilize the biological material by replacing the water molecules within the sample with molecules of the biopolymer. The cured polymer results in a solid protective film that is stable to many organic solvents, but quickly removed by the application of the water-based solution. The process of immobilization does not require the use of any additives, accelerators, or plastifiers and does not involve high temperature or radiation to promote polymerization.

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MINI-REVIEW

# Biopolymers for sample collection, protection, and preservation

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**Abstract** One of the principal challenges in the collection of biological samples from air, water, and soil matrices is that the target agents are not stable enough to be transferred from the collection point to the laboratory of choice without experiencing significant degradation and loss of viability. At present, there is no method to transport biological samples over considerable distances safely, efficiently, and cost-effectively without the use of ice or refrigeration. Current techniques of protection and preservation of biological materials have serious drawbacks. Many known techniques of preservation cause structural damages, so that biological materials lose their structural integrity and viability. We review applications of a novel bacterial preservation process, which is nontoxic and water soluble and allows for the storage of samples without refrigeration. The method is capable of protecting the biological sample from the effects of environment for extended periods of time and then allows for the easy release of these collected biological materials from the protective medium without structural or DNA damage. Strategies for sample collection, preservation, and shipment of bacterial, viral samples are described. The water-soluble polymer is used to immobilize the biological material by replacing the water molecules within the sample with molecules of the biopolymer. The cured polymer results in a solid protective film that is stable to many organic solvents, but quickly removed by the application of the water-based solution. The process of immobilization does not require the use of any additives, accelerators, or plastifiers and does not involve high temperature or radiation to promote polymerization.

**Keywords** Biopolymer · Acacia gum · Trehalose · Water · Soil · Air · Pathogens

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## Introduction

Contaminated air, water, and soil bring the threat to health, life, and property. Efficient and timely sample collection that allows transferring agents from the environment to detectors or laboratories is vital for prevention and environmental remediation of the agent exposure. A fundamental knowledge of sample collection from various matrices is crucial.

Recovery and preservation of microorganisms prior to analysis are important considerations of overall detection and identification schemes. Collection of suspected pathogens from the environment is usually based on air filtration or swab sampling followed by microbial analysis using traditional culture or PCR assay (Temprano et al. 2004). However, at least one study shows that only 10–13 % of bacterial strains could be recovered using these methods (Meunier et al. 2005). The main problem is that microbiological analysis first relies on adequate on-hand storage methods or immediate transport of microbes in the laboratory in order to ensure viability (Rutala et al. 2006). Current techniques of recovery and preservation have serious drawbacks, including structural damage to cell walls and degradation of nucleic acids (Beal et al. 2001;

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Broadbent and Lin 1999; Carvalho et al. 2004; Gouesbet et al. 2001). Consequences of freezing and freeze-drying include protein denaturation that leads to the decrease in cell viability (Carvalho et al. 2004; Leslie et al. 1995). The known processes of encapsulating immobilized bacteria in various starches involve spray-drying at elevated temperatures (Corcoran et al. 2004; Lian et al. 2002; O'Riordan et al. 2001) which is capable of damaging the microbes beyond repair. When preservation with conventional polymers is used, it usually involves elevating the temperature of the material or the use of UV radiation, both of which can again damage biological systems and nucleic acids. Resins are also used to some degree for the preservation of samples for electron microscopy, but the biological materials cannot be released for propagation after curing. Another method for encapsulating bacteria for use in biodegradation of gasoline involves a complex process using gellan gum (Moslemy et al. 2002). Many of these techniques have employed the use of protectants such as α-α trehalose, dimethyl sulfoxide (DMSO), glycerol, lactose, sucrose, and skim milk powder (Broadbent and Lin 1999; Carvalho et al. 2004; Conrad et al. 2000; Panoff et al. 2000), which contribute to the complexity of the preservation process. All of the aforementioned techniques are impractical for collecting samples in the field, are time-consuming, require special equipment and training, involve complex formulations, do not involve a broad spectrum of bacteria, and are limited in their applications.

Basic preservation methods, like refrigeration or tissue culture, have shortcomings such as limited shelf life, substantial cost, and possibility of contamination. As an alternative, cryopreservation has been effectively employed for long-term storage of biologicals. This method is founded on the concept that chemical, biological, and physical processes are adequately slowed down at cryogenic temperatures (−196 °C) (Mazur 1984). Usually, cryopreservation protocols use large concentrations (e.g., 1.0–2.0 M) of cryoprotectants like DMSO, glycerol, or ethylene glycol (Mazur 1984) simply because they easily pass through cell membranes and so provide protection to the intracellular components. However, cooling media that contain DMSO have been demonstrated to be toxic (Abrahamsen et al. 2002). A substitute to DMSO consists of nontoxic sugars such as trehalose. Trehalose is a nontoxic, nonreducing disaccharide of glucose that has a capability to stabilize and preserve cells and cellular components throughout freezing (Hino et al. 1990; Sano et al. 1999) and drying (Chen et al. 2001). The application of trehalose as a cryoprotectant may be related to its protective interactions with lipid membranes and proteins during freezing-thawing (Carpenter et al. 1986). An impediment to the application of trehalose as a cryoprotectant is its impermeability to mammalian cell membranes (Chen et al. 2001). Poration agents like ATP and benzoyl ATM need to be utilized to provide membrane permeability for trehalose (Buchanan et al. 2010).

Most of the existing methods of cell preservation are focused on the nucleic acid preservation and not on the safeguarding of cells (Gray et al. 2013). However, preservation of viable cells has definite advantages compared to the preservation of DNA, RNA only. First, the preserved cells could be multiplied by cultivation and provide large samples for testing, and second, live cells can also be used for physiological, pharmacological, and toxicological testing.

## Preservation with biopolymers

Acacia gum has been used for thousands of years. In Ancient Egypt, it was used as a pigment binder and adhesive in paints for making hieroglyphs, binder in cosmetics and inks, and for adhering flaxen wrappings for embalming mummies (Sanchez et al. 2002). In current times, acacia gum is used extensively in food industry as an emulsifier, flavoring agent/adjuvant, formulation aid, stabilizer/thickener, humectant, surface-finishing agent; in pharmaceutical industry as a suspending agent, emulsifier, adhesive, and binder in tableting and in demulcent syrups; in cosmetics as stabilizer in lotions and protective creams, and an adhesive agent in blusher; foam stabilizer in liquid soaps; in lithography; in textile industry; in painting as dispersant in paints; and as dispersant in insecticidal/ acaricidal emulsions (Baldwin et al. 1999; Joseleau and Ullmann 1990; Sanchez et al. 2002). We discovered that one of the most important applications is the use of acacia gum in preserving microorganisms for extended periods of time without refrigeration (Krumnow et al. 2009; Sorokulova et al. 2008, 2012).

The preservation with acacia gum involves a single compound and simple procedure. The process of immobilization in acacia gum does not include any additives, accelerators, or plastifiers, nor does it involve elevated temperatures or radiation to promote polymerization. The polymerization and replacement of water occur spontaneously in a single process. The method is reversible, and the solid polymer can be safely removed from bacteria.

Acacia gum (or gum arabic) is an exudate collected from *Acacia senegal* stems and branches (Baldwin et al. 1999; Joseleau and Ullmann 1990). Most of the acacia gum (90 %) obtained for commercial use comes from Sudan, Africa (Joseleau and Ullmann 1990). The exudate is a hydrocolloid composed of various molecular components of D-galactose (~40 %), L-arabinose (~24 %), L-rhamnose (~13 %), D-glucuronic acid (~21 %), 4-*O*-methyl-D-glucuronic acid (~2 %), and polypeptide (~2 %) (Michel et al. 1998; Mocak et al. 1998).

Large aggregates have been fractionated which include an arabino-galactan protein ($2.3 \times 106$ g $mol^{-1}$), arabino-galactan ($2.7 \times 105$ g $mol^{-1}$), and glycoproteins (Baldwin et al. 1999; Sanchez et al. 2002). The structural unit for these aggregates is "ß-(1,3)-linked galactose backbone with branches of β-(1,6)-linked galactose containing arabinose, rhamnose, uronic acids and their derivatives" (Sanchez et al. 2002). Acacia gum readily dissolves in cold and hot water in concentrations up to 50 % (JECFA 1998). The solutions are characterized by a low viscosity and pH range 4.5–5.5. This polymer is indigestible to both humans and animals, not degraded in the intestine, but fermented in the colon by microflora (Michel et al. 1998). It is biodegradable and has no ecological impairments.

Another natural biopolymer, trehalose, is often used for the preservation of biologicals. Trehalose is a stable, non-reducing disaccharide with two linked glucose molecules. Trehalose exists naturally in plants, animals, and microorganisms and has long been consumed by humans as a component of mushrooms, baker's and brewer's yeasts, seaweeds and such invertebrates as lobsters. Trehalose plays a vital role in the preservation of biomembranes and the revival of certain biological functions following desiccation or freezing (Teramoto et al. 2008).

Comparison of different techniques used for the preservation of biologicals at ambient temperature is given in Table 1. The preservation with a SampleMatrix® offered by Biomatrica®, San Diego, CA, is based on the proprietary synthetic compounds that mimic the protective properties of disaccharides, like trehalose (Lee et al. 2014). It is suitable for the preservation of DNA, RNA, and *Escherichia coli* strains only. It is not recommended for use with other bacterial species. DNAgard, another product of Biomatrica, was created to permeate the cells and preserve the DNA in a proprietary solution. This system does not require refrigeration or freezing to retain the stability of the DNA for long-term storage (Table 1) (Gray et al. 2013).

Recently, preservation of stem cells at room temperature for 4 weeks was demonstrated with trehalose (Buchanan et al. 2010). The method is very labor intensive and expensive. Beside the step of cell poration, this technique also includes a step of freeze-drying, which is not readily available in field conditions.

FTA technology (Whatman, GE Healthcare, Little Chalfont, UK) is based on the proprietary FTA cards that contain chemicals that lyse cells, denature proteins, and protect nucleic acids from damage (Table 1) (Gray et al. 2013). A solution-based mix of high salts, RNAlater® (Ambion, Carlsbad, CA) (Table 1), preserves tissues for a long time depending on the temperature. For extended storage, fresh samples are immersed in the solution, refrigerated overnight allowing saturation of the tissue, and frozen after that. RNAlater® has been demonstrated to

**Table 1** Comparison of cell and nucleic acid preservation techniques

| System | Medium | Preservation | Room temperature stability | Long-term storage | Reference |
|---|---|---|---|---|---|
| Acacia gum biopolymer | Biopolymer, dry | Gram-negative, Gram-positive bacteria, DNA, phages, antibodies, and proteins | Months, years | Room temperature | (Krumnow et al. 2009; Sorokulova et al. 2008, 2012) |
| Biomatrica® | SampleMatrix® | *E. coli* strains only. Not recommended for use with other bacterial species. DNA, RNA | 1 month for viable *E. coli* | Room temperature | (Biomatrica 2013) |
| Stabilizing sugar | Trehalose, liquid | Stem cells | 4 weeks | Room temperature | (Buchanan et al. 2010) |
| FTA® | Filter card | DNA | Years | Room temperature | (Gray et al. 2013) |
| FTA elute® | Filter card | DNA | Years | Room temperature | (Gray et al. 2013) |
| DESS | Liquid | DNA | Months | Room temperature | (Gray et al. 2013) |
| RNAlater® | Liquid | RNA | 1 week<br>1 month<br>Years | Room temperature, 4 °C–20 °C | (Gray et al. 2013) |
| DNAgard™ | Liquid | DNA | 1 month | Room temperature | (Gray et al. 2013) |

provide greater DNA yield than FTA® cards. RNAlater® furthermore gives the efficient preservation of RNA (Gray et al. 2013). DMSO/EDTA/saturated sodium chloride (DESS) is a nonproprietary solution that has been used to preserve DNA.

It was found that the liquid-based preservatives (DNAgard™, RNAlater®, and DESS) performed better than the card-based methods. Not one liquid technique evidently outperformed the others (Gray et al. 2013). An acacia gum biopolymer shows a very broad spectrum of preservation, including Gram-negative, Gram-positive bacteria, DNA, phages, antibodies, and proteins. It is also simple compared to other methods.

Among the methods available for assessing airborne viable particles (sieve/nozzle impactor, slit-to-agar, centrifugal, filtration, and impinge), the filtration may be the most suitable method for microbiological monitoring (Temprano et al. 2004). Specialized filters are the most suitable for the collection and analysis of airborne agents. Similarly, agents from water and soil are to be processed and filtered (Roger 1994).

Analysis of literature data allows us to conclude that method of preservation with acacia gum-based biopolymer is much more superior compared to other known to proposer's methods. It is simple, efficient, and cost-effective.

## Examples of preservation of microorganisms in natural biopolymers

Researchers used different methods to characterize the rate of the cell population reduction with time.

In 1943, (Katzin et al. 1943) defined the decimal reduction (D-value) time as follows:

$$D = \frac{2.3}{k} = t/\log\left(\frac{C_1}{C_2}\right) \quad (1)$$

where $k$ is the monomolecular reaction rate constant, and $C_1$ and $C_2$ are the initial and final concentrations of bacteria, respectively, subjected to a constant lethal temperature for $t=(t_2-t_1)$ duration of time. In this model, the classical D-value presents a simple biological significance: time that leads to a tenfold reduction of surviving population and is easily estimated from a simple linear regression. Other population reduction values can be readily converted to the D-value for comparison. The bacterial survival ratio (BSR) defined as $100\times\log C_1/\log C_2$ (Munoz-Rojas et al. 2006) can be transformed into the D-value of the following equation:

$$D = t_{\mathrm{BSR}}/\log C_1(1-\mathrm{BSR}/100) \quad (2)$$

The D-value is related to the time to degrade to 100 cells (Sorokulova et al. 2012), $t_{100}$ by the following expression:

$$D = t_{100}/(\log C_1 - 2) \quad (3)$$

The D-value calculated from the percent of survival $S=(C_2/C_1)\%$

$$D = t/\log\left(\frac{100}{S\%}\right) \quad (4)$$

The natural biopolymer, trehalose, is frequently utilized for the preservation of microorganisms. Trehalose significantly enhanced the tolerance of *E. coli* to air-drying (Table 1). The protective effects of trehalose during desiccation appear to be due to its stabilizing effect on membrane structure, its chemically inert nature, and the tendency of trehalose solutions to form glasses upon drying (Welsh and Herbert 1999).

The stability of a vacuum-dried *Lactobacillus paracasei* F19 was characterized by drying process in the presence of trehalose (Foerst et al. 2012). The D-value of the stored bacteria with trehalose was about four times larger compared with a D-value of unprotected cells (Table 2).

The protection capabilities of dry biopolymers were illustrated by the accelerated aging in alginate, POLYOX, carrageenan, and acacia gum (Rojas-Tapias et al. 2013, 2015). They reported storage enhancement of 1.8–5.5 (Table 2).

*Salmonella typhimurium* was immobilized in the solid polymer. After 21 days at room temperature, the bacteria were released and showed no difference in motility and viability compared to initial culture (Figure S1, and Preservation video, Supplementary Materials).

The formulations with *Bacillus subtilis* spores were tested for spore stability during storage at temperatures ranging from 40 to 90 °C and for bacterial release. Thermodynamic analysis showed that immobilization of spores in acacia gum significantly increased their viability compared with that of unprotected spores. The viability was further enhanced when suspensions of spores in acacia gum were added to the granules of charcoal (Table 2, Fig. 1). The number of the spores released after storage was also increased when spores were treated with acacia gum prior to immobilization in tapioca and charcoal. Formulations of *Bacillus* spores with acacia gum and porous carriers (charcoal and tapioca) prolong the anticipated shelf life of spores even under ambient temperature and provide slow and steady bacterial release consistent with their high viability (Sorokulova et al. 2008).

We demonstrated and characterize the efficacy of acacia gum biopolymer for preservation of pathogenic bacteria (*Bacillus anthracis* and methicillin-resistant *Staphylococcus aureus* (MRSA)) on different materials, used for swabbing and filtration: cotton, wool, polyester, rayon, charcoal cloth, and Whatman paper. The biopolymer used for the preservation of two pathogens has been shown to significantly

**Table 2** Preservation and storage of microorganisms immobilized in dry biopolymers

| Biopolymer | Microorganism | Temperature °C | D-value[a] | Reference |
|---|---|---|---|---|
| Trehalose | *E. coli* NCIB 9484, unprotected/protected | 25 | 3.3/36.3 days | (Welsh and Herbert 1999) |
| Trehalose | *Lactobacillus Paracasei* Unprotected/protected | 37 | 6.6/25.5 days | (Foerst et al. 2012) |
| Alginate | *Azotobacter chroococcum* Unprotected/protected | 15 | 6/23.7 days | (Rojas-Tapias et al. 2013, 2015) |
| POLYOX®[b] | *Azotobacter chroococcum* | 15 | 24.5 days | (Rojas-Tapias et al. 2013) |
| Carrageenan | *Azotobacter chroococcum* | 15 | 32.9 days | (Rojas-Tapias et al. 2013) |
| Acacia gum | *Azotobacter chroococcum* | 15 | 10.6 days | (Rojas-Tapias et al. 2013) |
| Acacia gum | *E. coli O1*:*K1*:*H7* (ATCC 11775) Unprotected/protected | 15 | 8/57.5 days | (Krumnow et al. 2009) |
| Pullulan | *E. coli O1*:*K1*:*H7* (*ATCC 11775*) Unprotected/protected | 15 | 8/33 days | (Krumnow et al. 2009) |
| Pullulan | *Bacillus subtilis*, ATCC 6051, humidity 76 % | 25 | 52.3 days | (Krumnow et al. 2009) |
| Acacia gum | *Bacillus subtilis*, ATCC 6051, humidity 76 % | 25 | 272 days | (Krumnow et al. 2009) |
| Acacia gum | *Bacillus subtilis*, ATCC 6051. Unprotected spores/Spores immobilized in charcoal+acacia gum | 20 | 1.6/48 years | (Sorokulova et al. 2008) |
| Acacia gum | *S. aureus* resistant to methicillin (MRSA) immobilized in filter paper/Filter paper+acacia gum | 15 | 43/89 days | (Sorokulova et al. 2012) |
| Acacia gum | *B. anthracis* Sterne spores, immobilized in filter paper/Filter paper+acacia gum | 50 | 1.1/3.1 years | (Sorokulova et al. 2012) |

[a] D-value is a time that leads to a tenfold reduction of surviving population

[b] POLYOX, water-soluble resins from Colorcon (Harleysville, USA)

protect bacteria during dehydration and storage in all tested samples at the range of temperatures (5–45 °C for MRSA and 40–90 °C for *B. anthracis*). Our results showed higher recovery as well as higher viability during the storage of both bacteria in all materials with acacia gum (Table 2). The addition of acacia gum polymer to swabbing materials or filters will increase the efficacy of sample collection and identification of pathogenic bacteria from locations such as hospitals or the environment. The approach can also be used for long-term storage of culture collections since acacia gum contributes to the viability and stability of bacterial cultures (Sorokulova et al. 2012).

Natural polymers of acacia gum and pullulan were used to preserve model bacteria *E. coli* and *B. subtilis* via immobilization and storage under various conditions. Formulation of *E. coli* and *B. subtilis* in acacia gum significantly increased the viability of both cultures during desiccation at 40 °C as well as during the storage at various temperatures and relative humidity. In the ranges of temperatures and humidity used in experiments, the high humidity affected the viability of *E. coli* more than high temperature. Thermodynamic parameters for *E. coli* thermal degradation were used for quantification of the results and characterization of the preservation process. The viability of *B. subtilis* in the acacia gum polymer was not significantly changed during the storage in the temperature and humidity experiments. The number of viable *B. subtilis* B. subtilis recovered after storage in pullulan, and in PBS under various humidity conditions, was 1–2 logs less in comparison with the number of cells before storage. It was found that acacia gum provides better protection than pullulan for both bacteria during the preservation process (Table 2) (Krumnow et al. 2009).

## Sampling and preservation strategies

### Standard and enhanced procedures for sampling collection

Stability of microbial cultures during sampling and storage is a vital issue in various fields of medicine, biotechnology, food science, and forensics. Acacia gum biopolymer-based preservation has many advantages. It is a simple and inexpensive process that does not require specialized equipment or training to preserve bacteria successfully. The most important application of the biopolymer is collecting samples in the field. It has been shown in our preliminary studies that acacia gum is capable of preserving bacteria in stringent conditions such as high temperature and humidity without refrigeration. Thus,

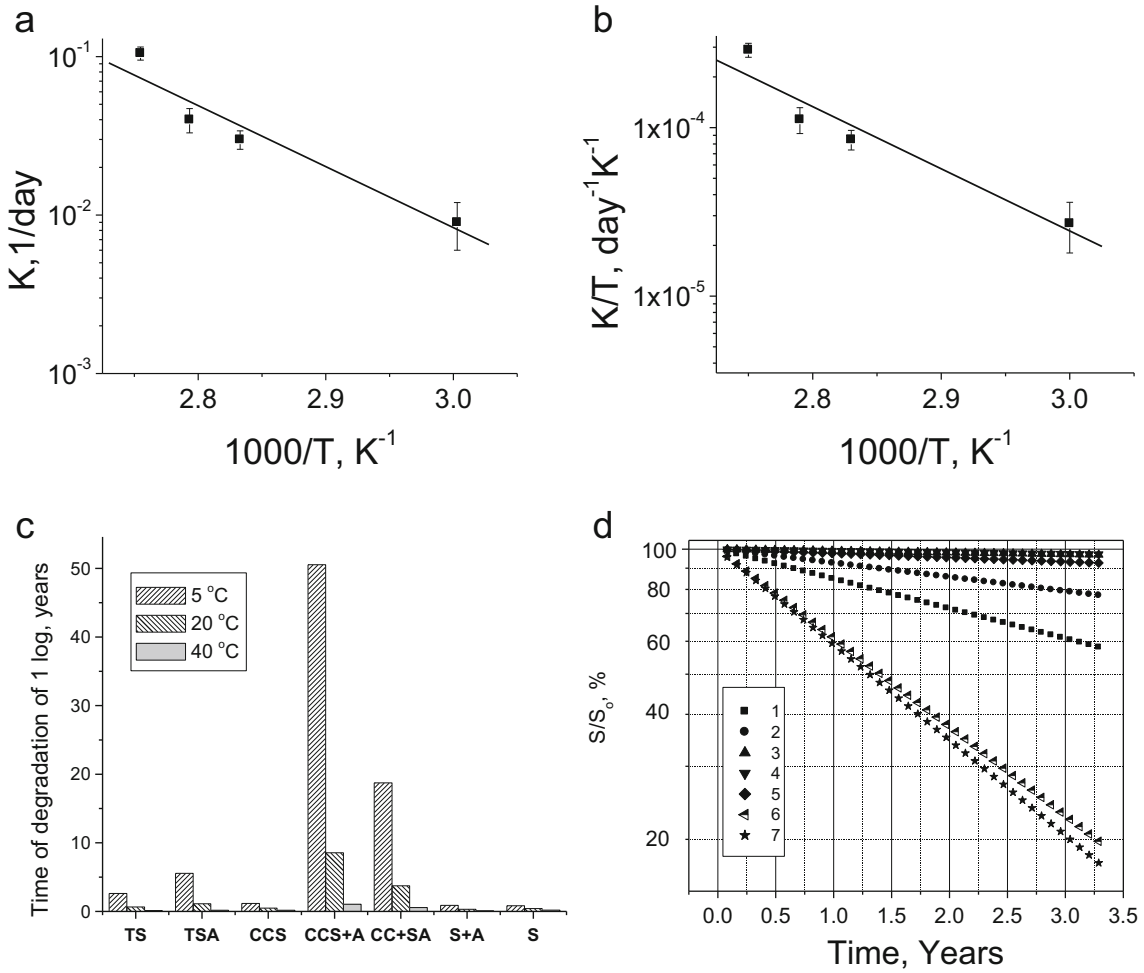


**Fig. 1** The estimated viability of spores by Arrhenius and Eyring equations. **a** *Points* are experimental data plotted by Arrhenius plot; the *line* is a linear regressions: $R$ ($P$)=−0.96 (<0.04). **b** *Points* are experimental data plotted by Eyring equation, and the *line* is a linear regressions. $R$ ($P$)=−0.96 (<0.05). **c** Time for degradation of one log of spores at different temperatures. **d** The viability of spores (percent of surviving spores, $S/S_o$ %) at 5 °C; *1*—tapioca+spores (*TS*); *2*—tapioca+(spores+acacia gum) (*T+SA*); *3*—charcoal+spores (*CCS*); *4*—(charcoal+spores)+acacia gum (*CCS+A*); *5*—charcoal+(spores+acacia gum) (*CC+SA*); *6*—spores+acacia gum (SA); *7*—spores (*S*) (from Sorokulova et al. 2008, by permission from WILEY # 3603090702078)

collecting samples at remote locations using biopolymer may be applied to preserving microorganisms for further testing in a laboratory. Normally, the smaller the time that elapses between the collection of a sample and its examination, the more reliable will be the analytical results. The Table 1 shows that acacia gum biopolymer is the most effective compared to other cell preservation methods. Additionally, the acacia gum biopolymer is the only one that was examined to preserve a large variety of biological.

The standard procedure followed the environmental sample collection that includes first refrigeration by ice packs, dry ice, or portable refrigerator, then transportation and storage, and finally the laboratory analysis (Kelly et al. 2006; NEWWA 2008; ODH 2008). The enhanced method of the sample collection replaces the step of refrigeration with the preservation utilizing the acacia gum biopolymer. The enhancement of the modified method can be illustrated by a simple example. If one collects 1000 water samples according to a general First Responder Procedure, water tubes with samples are cooled to +4 °C with ice or refrigeration and transferred to the laboratory for storage and testing. The volume of the sampling containers may be 40 mL, 60 mL, or 1 L, depending on the analytical method used (NEWWA 2008). Cooler boxes need to be filled with ice to have ice to sample ratio of at least 2:1 to achieve an ice retention for about 3–4 days (YETI 2013). The price of a single 60-mL sample is a sum of the costs of a bottle, ice, freight transportation, and storage. Five percent of a container weight needs to be added to a weight of a sample. Using acacia gum biopolymer method, a nonwater content of 60 mL sample is filtered. The filter with microorganisms is immobilized in biopolymer and transferred to a test location in a small zip bag. Table 3 compares the general method and the method that utilizes the acacia gum biopolymer for preservation during the transport and

**Table 3** Comparison of sample collection price and efficiency by different methods

| Properties | Methods | | Gain by the proposed method |
|---|---|---|---|
| | Standard | Acacia gum biopolymer | |
| Disposables[a], $ per sample | 0.72 | 0.78 | 0.92 |
| Ice[b]/biopolymer[c], $ per sample | 0.048 | 0.01 | 4.8 |
| US Freight by truck[d], 1000 miles, $ per sample | 0.015 | 0.000074 | 200 |
| Efficiency[e], $ per sample per day | 0.0979 | 0.0376 | 2.6 |

[a] 60 mL bottles (Dynalon 2011) are used in the standard method. In the biopolymer method, 2×3 zipper seal bags (Dynalon 2011), filters (VWR #28157-949), and Petri dishes (VWR #82050-536)

[b] $0.4 is a price of 1 kg of ice. One sample requires 120 g of ice, and it costs $0.048

[c] The cost of biopolymer in one sample is $0.01 (Sigma-Aldrich #G9752)

[d] It takes 3357 BTU to move 1 t 1000 miles (Davis et al. 2014), and it costs $21.98 per million BTU (EIA 2012). The weight of one cooled sample in the standard method ($W1$) is comprised of the weight of the empty bottle, sample, ice, and container. $W1=13+60+120+9.7\approx 204$ g. The sample weight in the biopolymer method ($W2$) is comprised of the weight of the empty bag, filter, solid biopolymer, ice, and container. $W2=0.4+0.1+0.2+0.35\approx 1$ g. Therefore, US Freight by truck, 1 mile for the standard method costs $=3357\times 204\times \$21.98\times 1.10^{-9}=\$0.015$, while in the proposed method costs $=3357\times 1\times \$21.98\times 1.10^{-9}\approx \$0.000074$.

[e] The efficiency of sample collection takes into account a viability loss during sampling, transfer, and storage. The higher the number of viable microorganisms, the greater efficiency of sampling can be. The efficiency is exemplified by a sampling of *E. coli*. At 15 °C, D-values with and without biopolymer are 57 and 8 days, respectively, (Table 2). The efficiency is calculated as total price per sample/D-value

storage before tests. The sampling efficiency of particular microorganism is defined by the price of sampling per sample per D-value of the microorganism.

As can be seen from the Table 3, this preservation system significantly improves the performance of the environmental sampling. The collected samples need no refrigeration for transport and storage. The weight of collected samples is dramatically reduced. One thousand 60 mL samples on ice weigh 204 kg, while the same number samples preserved by biopolymer weigh only 1 kg. Therefore, the freight is about 200 less expensive. The efficiency of sampling (the price of collecting and transfer viable bacteria) is strongly increased. As exemplified by sampling of *E. coli* in water, the sampling efficiency with biopolymer is increased by 260 %. The use of filters as a carrier for biopolymer prolongs the viability of microorganisms at the average by four times (Krumnow et al. 2009). Therefore, the efficiency of sampling with carrier filters and biopolymer may over-perform the standard method by more than ten times.

### Concept of preservation at ambient temperature by biopolymer

Acacia gum biopolymer is a natural product that can preserve cells and other biologicals for extended periods at room temperature. Current methods require cold temperatures, which are labor intensive, expensive to maintain, and make the samples difficult to transport. The biopolymer preserves embed samples in a stable, protective film. Samples can be recovered by simply adding water, without the need for heat or radiation. Samples have been kept alive for over 6 months at room temperature and over 16 months at refrigerated temperatures (Krumnow et al. 2009; Sorokulova et al. 2008, 2012)

The biopolymer technology is applied in three stages:

1. Immobilization and polymerization
   - Biopolymer provides structural integrity, guards against mechanical stress, and slows metabolic processes in cells.
2. Stabilized state existence (dormant state for cells)
   - The dormant state is characterized by slow metabolism, water replacement; partial water retention; and molecular stability.
3. Release
   - Biopolymer protects against structural changes, releases mechanical stress, and facilitates the increase of metabolism in cells.

## Microorganism's collection and preservation schemes for contaminated air, water, and soil

### Air samples

The samples of the environmental air are collected by filtering relatively large volumes of air drove by a battery-driven

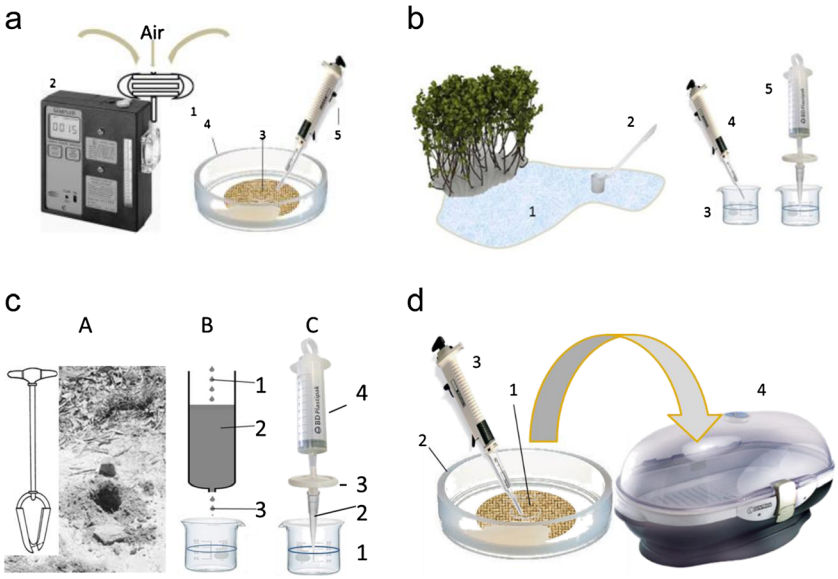


**Fig. 2** Sample collection from the environment. **a** Sample collection of environmental air. *1*—filter cassette with filter, *2*—portable battery-driven pump, *3*—filter with collected bacteria from ambient air, *4*—Petri dish, *5*—micropipette with liquid biopolymer. **b** Sample collection of environmental water. *1*—body of water, *2*—dipper, *3*—beaker with collected environmental water, *4*—syringe filtration system. **c** Collection of environmental soil samples. *A* represents the collection of soil samples with an auger. *B* represents the microorganism elution (*1*—water, *2*—soil, *3*—suspension of microorganisms). *C* represents the collection of microorganisms by syringe filtration. **d** Preservation of microorganisms with biopolymer. *1*—filter with collected microorganisms, *2*—Petri dish, *3*—micropipette with liquid biopolymer, *4*—battery-driven potable desiccator

portable pump as shown in Fig. 2a. The filter with trapped environmental microorganisms (3) is positioned at the bottom of the sterilized Petri dish (4) and added liquid biopolymer. After the last step, the sample is ready for polymerization.

### Water samples

The environmental samples of water are collected from a natural body of water (1) using a dipper (2) as shown in Fig. 2b. The sample is added to the volumetric beaker (3) and filtered through a syringe filter system (4). After filtration, the filter with collected microorganisms is covered with liquid biopolymer.

### Soil samples

The soil samples are collected from the environmental soil (Fig. 2c A). The samples then are inoculated by the sterile dH2O (Fig. 2c B) and, finally, subjected to filtration and collection by the filter (Fig. 2c C). After that, the filter with the agent will be immobilized in biopolymer.

### Sample polymerization

After microorganisms are collected on a filter, and each filter is positioned in the individual small Petri dishes, covered with liquid biopolymer, the Petri dishes with samples then are covered with vented covers and transferred to the portable battery-driven desiccator to dry and polymerize for ~2 h. After that, the samples are ready for storage and analytical tests. The control filters with microorganisms are also going through drying in a desiccator (Fig. 2d).

## Release of microorganisms and DNA analysis

After samples arrive at the laboratory, microorganisms are released and analyzed. Every sample can be placed in 10 mL of water buffer solution, homogenized, and filtered. The filtrate containing microorganisms is subjected to DNA analysis (Ogram et al. 1987; Park and Crowley 2005; Zhou et al. 1996).

## Conclusions

The method of collection and preservation of microorganisms with the use of water-soluble biopolymers has a few advantages as compared with standard methods utilizing refrigeration.

- Immobilization of microorganisms in dry biopolymer allows the collection of biological samples from air, water, and soil matrices and transfers them from the collection

point to the laboratory of choice without experiencing significant degradation and loss of viability.

- Dry biopolymers are capable of preserving biological samples for extended periods (30+days) without refrigeration.
- Water-soluble biopolymers ensure the complete recovery of preserved biological materials.
- The method of preservation with dry biopolymers is simple and rapid.
- Collected biological specimens are released from the biopolymer by the water-based solution.
- Hardens/solidifies under simple conditions (no temperature above 40 °C, no chemicals or radiation).
- Nontoxic and environmentally friendly.
- Economically sound.
- Acacia gum does not interfere with established sample examination techniques (microscopy, PCR, pulsed-field electrophoresis, and other methods.)
- Formulations of microorganisms with biopolymer and porous carriers (charcoal and tapioca) prolong the shelf life under ambient temperature and provide slow and steady release.
- Filters made of cotton, wool, polyester, rayon, charcoal cloth, and Whatman paper impregnated with acacia gum were shown to enhance the preservation of pathogenic bacteria actively.

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**Conflict of interest** The authors declare that they have no competing interests.

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