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Evaluation of New Components in Modified Scholten's Medium for the Detection of Somatic Coliphages

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Abstract

Enteric bacteriophages (somatic coliphages, F-specific coliphages or both together) are now recognized as useful viral indicators in water, shellfish, and biosolids and are being progressively included in national and international sanitary regulations. Among them, somatic coliphages have an advantage in that they usually outnumber F-RNA coliphages in water environments. Their enumeration using Modified Scholten's (MS) media, following the ISO 10705-2 standard for the growth of *Escherichia coli* host strain WG5, is highly efficient and a common practice worldwide. These media contain a high concentration of nutrients, which may be modified to save costs without loss of bacterial growth host efficiency. This study explored reducing the concentration of nutrients in the current formulation and/or incorporating new components to improve the host

bacterial growth and/or the enumeration of somatic coliphages at an affordable analytical cost. A twofold dilution of the original MS media was found not to affect the bacterial growth rate. The addition of combinations of assayed compounds to twofold diluted MS media slightly enhanced its analytical performance without altering bacterial growth. By generating savings in both cost and time while maintaining optimal results, media dilution could be applied to design new simple applications for coliphage enumeration.

Keywords

Somatic coliphages
Bacteriophage
Viral indicators
Water quality
Culture medium

Introduction

For more than a century, the evaluation of the microbiological quality of water has been based mainly on bacterial indicators (Feachem et al. 1983; Ashbolt et al. 2001). After progressive improvements in specific and practical applications and analytical techniques such as membrane filtration methods, defined substrate methods, or microbial source tracking (Ashbolt et al. 2001; Farnleitner and Blanch 2017), bacterial indicators are a key contributor to the control of waterborne infectious diseases. However, bacteria have certain limitations, for example, they are not always representative when used as indicators of pathogenic viruses in certain situations and water treatments in both developed and developing countries (Borchardt et al. 2004; Gerba et al. 1979; Grabow 2001; Keswick et al. 1984; Payment et al. 1997). As an alternative, in the last decades, enteric bacteriophages have been proposed as viral indicators in water as well as food and biosolids (Havelaar et al. 1986; Hsu et al. 2002; Jofre 2007; Kott et al. 1974; WHO 2017a, b).

Enteric bacteriophages are obligate intracellular microorganisms (Adams 1959) that are abundant in the guts of animals, infect specific enteric bacteria, and generally do not replicate in the environment (Brion et al. 2002; Muniesa and Jofre 2004). Three main groups of bacteriophages have been proposed as improved indicators for viral fecal pollution: somatic coliphages (infecting *Escherichia coli* through the cell wall), F-specific phages (infecting *E. coli* and other enterobacteria through the sex pili encoded by the F-plasmid), and phages of *Bacteroides* spp. (infecting host strains through the cell wall). Standardized methods for the detection of all the bacteriophage groups (ISO 10705-1, ISO 10705-2, US-EPA 1601, US-EPA 1602) (Anonymous 1995, 2000; Anonymous 2003a, b, c) and procedures for validating methods for the concentration of bacteriophages from water (ISO 10705-3) (Anonymous 2003a, b, c) have been developed.

The utility of somatic coliphages, F-specific coliphages, or both together (total coliphages) has been increasingly acknowledged and they are now included as viral indicators for water, shellfish, and biosolids in national and international sanitary regulations (NHMRC and NRMMC 2011; North Carolina Administration 2011; Queensland Government 2005; Republica de Colombia 2014; Republique Française 2014; U.S. EPA. 2016; U.S. EPA 2015; Western Australian Government 2012; Food and Drug Administration and Interstate Shellfish Sanitation Comission 2015). The European Commission recently included coliphages among microbial parameters in a new EU drinking water directive (https://ec.europa.eu/info/law/better-regulation/initiatives/com-2017-753_en) and for the validation of treatment processes in water reuse (https://ec.europa.eu/info/law/better-regulation/initiatives/com-2018-337_en). Scientific reviews, meta-analysis studies, and guidelines also support their use as viral indicators in water monitoring and management practices for different types of water use (U.S. EPA 2015; WHO 2017a; Nappier et al. 2019).

Somatic coliphages usually outnumber F-RNA phages in water environments (Jofre et al. 2016; Mooijman et al. 2005; WHO 2017b; Nappier et al. 2019). They have also been previously reported as good indicators for viral fecal pollution (Armon and Kott 1996; IAWPRC Study group on health related water microbiology 1991; Jofre 2007).Their enumeration using Modified Scholten's (MS) media (MSB: Modified Scholten's Broth; ssMSA: semi-solid Modified Scholten's Agar; MSA: Modified Scholten's Agar) according to the ISO 10705-2 standard (Anonymous 2000) is commonly performed in conventional laboratories worldwide. These MS media are formulated with a high concentration of nutrients and have proved highly efficient for bacterial host growth and subsequent enumeration of somatic coliphages. Nevertheless, the incorporation of new components used in other culture media for the growth of *E. coli* strains, and/or a reduction of the nutrients in the MS media may improve (i) the growth of the host strain *E. coli* WG5 (ISO 10705-2), providing an optimal layer of bacterial growth, (ii) the recovery of somatic coliphages for their enumeration, and (iii) the costs of the media used for coliphage determination.

In this study, the currently used MS media were modified by incorporating several compounds found in other culture media formulations for *E. coli* and by removing certain components, and the performance of the new medium was assessed.

Material and Methods

Bacteriophages and Bacterial Strains

A pure culture of the somatic coliphage ΦX174 (ATCC 13,706-B1) in laboratory stocks of known concentration (10^9 plaque-forming units/mL) was used as a reference bacteriophage in accordance with the ISO 10705-2 standard. *E. coli* strain WG5 (ATCC 700078) was used as a host strain for somatic coliphages as described in the ISO 10705-2

standard (Anonymous 2000) in assays of growth kinetics and medium optimization by enumeration of colony-forming units (CFU) and plaque-forming units (PFU), respectively.

Medium and Growth Conditions

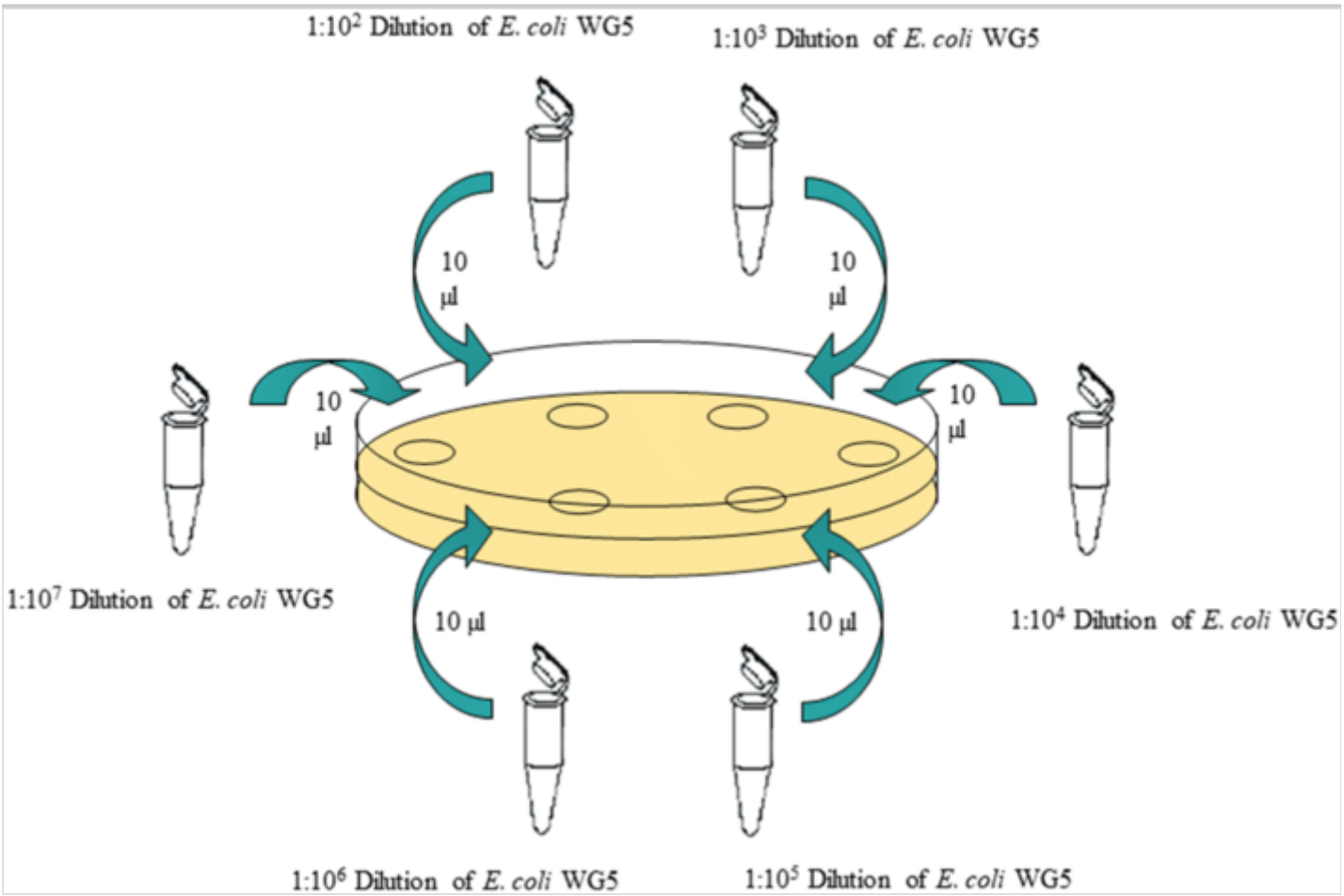
All the studied media formulations (solid, semi-solid or broth) were used following the established conditions in the ISO 10705-2 standard for the culture of *E. coli* WG5 strain (Anonymous 2000). MSB, MSAss, and MSA were used to set up the base values for bacterial growth and later for the enumeration of somatic bacteriophages applying the double agar layer (DAL) method as indicated in the ISO procedure. According to ISO 10705-2, the MSB components are: 10 g/L of peptone, 3 g/L of yeast extract, 12 g/L of meat extract, and 3 g/L of NaCl. For the preparation of MSA, 15 g/L of agar is added to the composition, and for MSAss, only 7.5 g/L of agar is added. Additionally, 5 mL of Na_2CO_3 (150 g/L) and 0.3 mL of $MgCl_2$ (4.14 M) per liter were always added before sterilization. After the sterilization process, the pH was adjusted to 7.0–7.5. Incubation of all media formulations was always performed at 37 ℃ for 18 h to quantify bacterial growth (by CFU) and somatic coliphages (by PFU).

Colony-Forming Unit Enumeration in the Experimental Media

The growth of *E. coli* strain WG5 in the media containing new compounds was initially assessed by enumerating the CFU using the Miles–Misra–Irwin method (Miles et al.1938). This consists of making tenfold serial dilutions of a fresh bacterial culture and plating one drop of 10 µL of each dilution in an agar plate of the assayed medium. For every designed medium, six dilutions (from 10^{-2} to 10^{-7}) were inoculated on each plate, each with five replicates (See Fig. 1). MSB was used to prepare the fresh culture of *E. coli* strain WG5. A positive control of MSA was used as the base level reference for statistical analysis of significances for the new media in each assay. For every experiment performed with the new media, a negative control using sterile MSB was also plated.

Fig. 1

Schematic representation of Miles–Misra–Irwin method applied for CFU/mL enumeration



Bacterial Growth Kinetics of the Assayed Media

The kinetics of bacterial growth were measured by spectrophotometry, observing the increase of optical density at 600 nm (OD_{600}). Growth curves were monitored every 30 min until the culture reached the cell density of > 10^8 CFU/mL, when exponential growth stopped. According to ISO 10705-2, an exponential phase culture of the bacterial host is needed for bacteriophage enumeration, the main aim of this study.

AQ1

Plaque-Forming Unit Enumeration in the Experimental Media

PFU of somatic coliphages were counted by the DAL technique and using a host strain *E. coli* WG5 as described in the ISO 10705-2 standard (Anonymous 2000). The DAL technique consists of several steps in which a sample containing bacteriophages, a host strain, and semi-solid agar medium are mixed and plated on MSA plates forming an overlay. Enumeration of PFU was performed after 18–24 h of incubation at 37 °C. In this instance, MS media and ½ MS media were used for the DAL as described in the ISO 10705-2 standard for the detection of a calibrated suspension of the somatic coliphage ΦX174.

New Compounds and Concentrations

Different compounds were added to the MS media to evaluate if they could improve the bacterial growth and/or the enumeration of somatic coliphages. Their selection was based on the composition of other selective media for *E. coli* such as Chromocult, Difco MI, DifcoMTEC, Colilert, and HardychromEsBL (Atlas 2010). Eight compounds were chosen, each used at two concentrations (Table 1) according to the usual concentrations in other enterobacteria growth media: casamino acids (CAS 91079-40-2), sodium pyruvate (CAS 113-24-6), tryptophan (CAS 73-22-3), thiamine (CAS 67-03-8, sodium lauryl sulfate (CAS 151-21-3), sodium deoxycholate (CAS 302-95-4), disodium hydrogen phosphate dodecahydrate (CAS 10039-32-4), and sodium dihydrogen phosphate (CAS 7558-80-7).

Table 1

Selected components in the initial design and their concentrations

Component	Low concentration (L) (g/L)	High concentration (H) (g/L)
Casamino acids	1.600	8.000
Sodium pyruvate	0.005	1.000
Tryptophan	0.005	1.000
Thiamine	–	0.026
Sodium lauryl sulphate	–	0.200
Sodium deoxycholate	–	0.100
Disodium phosphate	–	2.700
Sodium dihydrogen phosphate	–	2.200

The significance of the effects of the selected eight compounds at two concentrations was determined using the statistical model 2^k (Christen and Raimbault 1991; Davies 1993), which analyzes the importance of k factors (compounds) in a determinate event (medium definition). Consequently, eight factors ($k = 8$) were used for the evaluation, each at two concentrations. These parameters allowed the definition of 2^8 media. In order to minimize the analyses, a folded-over Plackett–Burman design was performed (Plackett and Burman 1946), with a run size of $N = 24$ of up to 8 factors. A Plackett–Burman design is a type of screening design that facilitates finding out which factors in an experiment are important, allowing the analysis of a great number of factors without performing a complete factorial design. Therefore, it is a great tool for an initial screening for which factors to concentrate on in the following experiments. Thus, the number of potential media was reduced from 2^8 (factorial design) to 24 combinations with five replicates of each in the Plackett–Burman design. The bacterial strain *E. coli* WG5 was used to evaluate all these media by the enumeration of total CFU. A McFarland no. 3 cell suspension (McFarland 1907) in 0.25× PBS solution was prepared after growing the strain on MSA for 48 h. This suspension used as a reference to adjust the turbidity of bacterial suspensions has a turbidity equal to 9×10^8 CFU/mL of bacterial growth. Tenfold dilutions were made from these suspensions

in 0.25× PBS solution. Five replicates of plating for each experimental medium were performed following the Miles–Misra–Irwin method (Miles et al. 1938) which is described in Sect. 2.3 of this article.

From the Plackett–Burman design three compounds were observed to improve significantly bacterial growth in Miles–Misra–Irwin assays. These components were selected for a full factorial design using three different concentrations of each, giving 3^3 possible combinations, in order to determine the optimal concentrations and possible synergies between them. The selected compounds were sodium lauryl sulfate (CAS 151-21-3) [L0 = 0 g/L of sodium lauryl sulfate; L0.5 = 0.1 g/L of sodium lauryl sulfate; L1 = 0.2 g/L of sodium lauryl sulfate], sodium deoxycholate (CAS 302-95-4) [D0 = 0 g/L of sodium deoxycholate; D0.5 = 0.05 g/L of sodium deoxycholate; D1 = 0.1 g/L of sodium deoxycholate] and disodium hydrogen phosphate dodecahydrate (CAS 10039-32-4) [H0 = 0 g/L of disodium hydrogen phosphate dodecahydrate; H0.5 = 1.35 g/L of disodium hydrogen phosphate dodecahydrate; H1 = 2.7 g/L of disodium hydrogen phosphate dodecahydrate]. Once again, bacterial growth was measured using the Miles–Misra–Irwin method as previously described. Combinations of the selected compounds which yielded better CFU/mL results were subjected to further analysis.

After analyzing the initially chosen eight compounds, an additional compound, sodium propionate, was independently assayed at three concentrations with the same methodology. Used in the food industry for inhibiting bacterial growth, sodium propionate was tested to see if it would facilitate CFU enumeration besides inhibiting the growth of other microorganisms. This would be of interest, as it could contribute to saving time by avoiding the filtration step in ISO guidelines for eliminating accompanying microbiota (Anonymous 2000). Concentrations were the following: sodium propionate (CAS 137-40-6) [P1 = 1 g/L of sodium propionate; P2 = 2 g/L of sodium propionate; P4 = 4 g/L of sodium propionate].

Subsequently, twofold, threefold, fourfold, eightfold, 16-fold, and 32-fold diluted MS media were tested using the Miles–Misra–Irwin method (Miles et al. 1938) for CFU/mL determination, the measurement of OD_{600nm} for growth rate comparison, and the DAL method according to ISO 10705-2 (Anonymous 2000) for PFU/mL enumeration of somatic coliphages. This approach would allow us to determine the feasibility of reducing the concentrations of nutritional components of the established MS media without negatively affecting bacterial growth or bacteriophage detection. The original MS media composition was used as a control in all analyses.

The most favorable combinations of new components and dilutions of the existing ones providing similar results to the original media were assayed for growth rate (OD_{600nm} measurement) and bacteriophage detection (PFU/mL enumeration by the DAL method) to determine the most optimal combinations.

Statistical Analysis

Experimental design, computation of data, and statistical tests were performed using Statgraphics software (Statgraphics Centurion XVI v.16.0.07) (Statpoint Technologies, Inc.). ANOVA analysis with 95% confidence intervals was performed to corroborate the significance of differences observed in the obtained data. *E. coli* and somatic coliphage concentrations were log10 transformed to normalize the data prior to statistical analysis.

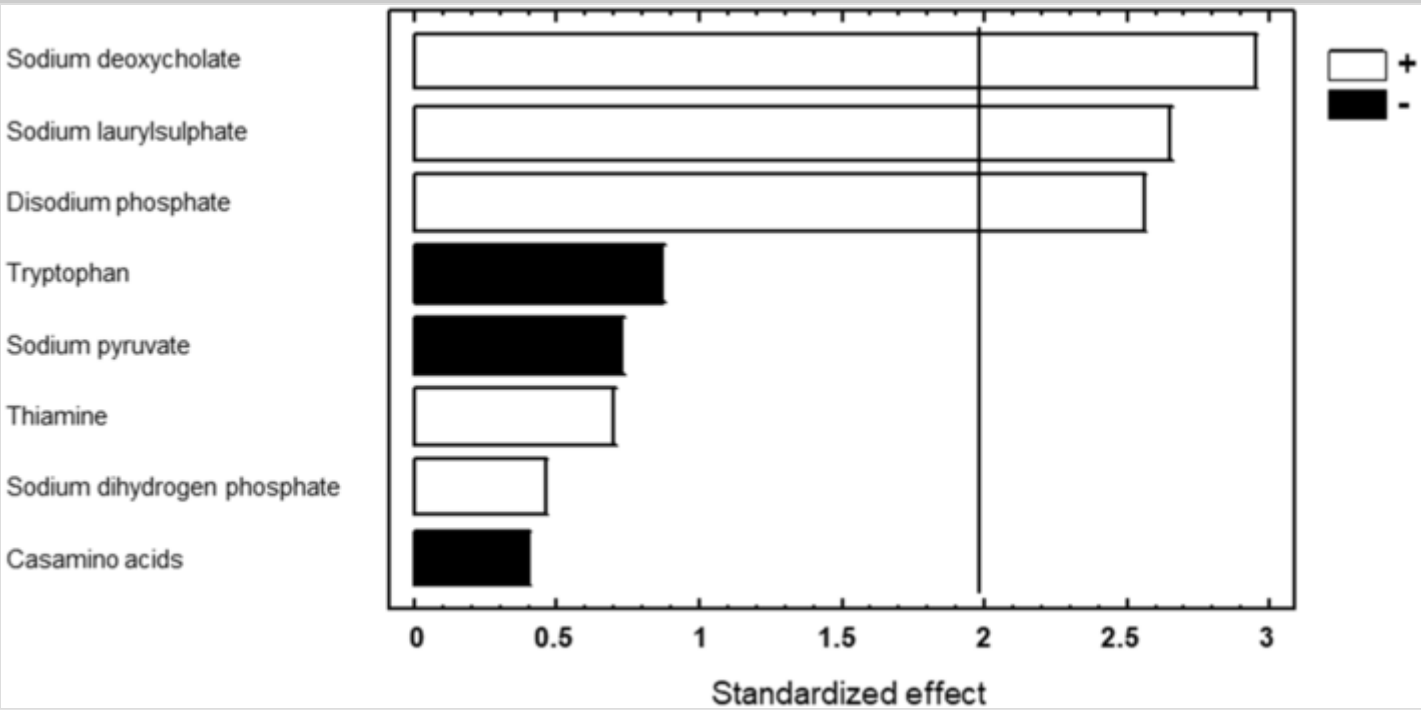
Results

Colony-Forming Unit Enumeration in the Experimental Media

The quantification of bacterial growth using the Miles–Misra–Irwin method (Miles et al. 1938) based on the initial factorial design showed differences in growth among the studied compounds. Standardized effects of each component added to the medium are represented in Fig. 2. This Pareto chart shows the absolute values of the standardized effects from the largest to the smallest. The standardized effects are t-statistics that test the null hypothesis (zero effect). The chart also plots a reference line to indicate which effects are statistically significant considering a significance level of $\alpha = 0.05$, for determining significance in the Pareto chart with 95% confidence intervals. The factors with bar extending beyond the vertical line on the pareto chart shows significant influence at 95% confidence level. Three of the eight assayed components (sodium deoxycholate (p-value = 0.0039), sodium lauryl sulfate (p-value = 0.0093), and disodium hydrogen phosphate dodecahydrate (p-value = 0.0119)) surpassed this threshold (Fig. 2), thus having a positive influence on bacterial growth. The other 5 components had either a negative effect on growth or a small positive effect that did not reach the significance threshold.

Fig. 2

Standardized effect pareto chart for bacterial growth measurements (CFU/mL) obtained in Miles–Misra–Irwin assays for the eight studied compounds, marked with (+) if their presence had a positive effect overall and (–) for negative effects. Vertical line represents a confidence interval of 95%



The subsequent experimental design testing these three potentially favorable components yielded better results in bacterial growth in the following combinations (Table 2): L0_D0_H0.5; L0_D0_H1 and L0_D1_H1 (L0 = 0 g/L of sodium lauryl sulfate, D0 = 0 g/L of sodium deoxycholate, D1 = 0.1 g/L of sodium deoxycholate, H0.5 = 1.35 g/L of disodium hydrogen phosphate dodecahydrate; H1 = 2.7 g/L of disodium hydrogen phosphate dodecahydrate).

Table 2

Selected media CFU/mL counts and component concentrations (g/L) in each of them

Medium	MSB concentration (g/L)	Sodium deoxycholate concentration (g/L)	Disodium phosphate concentration (g/L)	Sodium propionate concentration (g/L)	CFU/mL	SD
MSB	28	–	–	–	6.09×10^8	2.8×10^7
MSB L0_D0_H0.5	28	–	1.350	–	6.31×10^8	3.2×10^7
MSB L0_D0_H1	28	–	2.700	–	6.43×10^8	3.3×10^7
MSB L0_D1_H1	28	0.100	2.700	–	6.40×10^8	1.1×10^7

L sodium lauryl sulfate, *D* sodium deoxycholate, *H* disodium hydrogen phosphate dodecahydrate, *P* propionate

Medium	MSB concentration (g/L)	Sodium deoxycholate concentration (g/L)	Disodium phosphate concentration (g/L)	Sodium propionate concentration (g/L)	CFU/mL	SD
MSB P4	28	–	–	4.000	6.57×10^8	2.3×10^7
MSB 1/2	14	–	–	–	6.01×10^8	2.7×10^7
MSB 1/2 L0_D0_H0.5	14	–	1.350	–	6.07×10^8	3.0×10^7
MSB 1/2 L0_D0_H1	14	–	2.700	–	6.19×10^8	3.2×10^7
MSB 1/2 L0_D1_H1	14	0.100	2.700	–	6.16×10^8	1.1×10^7
MSB ½ P4	14	–	–	4.000	6.45×10^8	2.3×10^7

L sodium lauryl sulfate, *D* sodium deoxycholate, *H* disodium hydrogen phosphate dodecahydrate, *P* propionate

A similar analysis with the potentially inhibitory component (sodium propionate) was performed independently. Of the concentrations assayed, P4 (4 g/L of sodium propionate added) was the only one that performed significantly better than the original medium (*p*-value = 0.023).

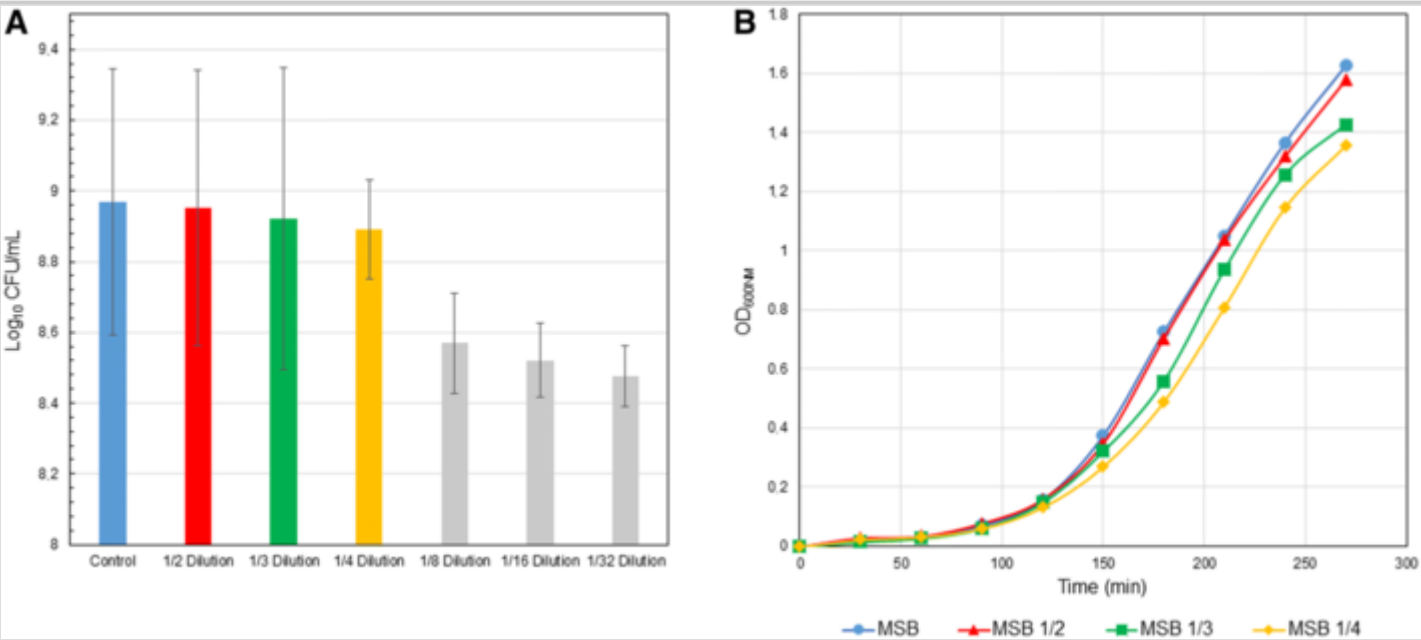
Altogether, among the designed media, those that yielded a better bacterial growth were MSA supplemented with the following compounds and concentrations: L0_D0_H0.5; L0_D0_H1; L0_D1_H1, and P4 (Table 2). These four higher performing combinations were selected for further analyses of bacteriophage enumeration and bacterial growth curve assays.

Concentration of the Original MS Medium

The bacterial growth measurements were assayed in MS and several diluted MS media. Results of *E. coli* WG5 growth showed that the original formulation of this medium contains an excessively high concentration of nutrients (Fig. 3a). Thus, the original MS medium can be diluted up to fourfold without causing any significant effect on bacterial growth (1/2 *p*-value = 0.828, 1/3 *p*-value = 0.571, 1/4 *p*-value = 0.236). Higher dilutions of the MS media were significantly detrimental for bacterial growth. (1/8 *p*-value = 3.63E-04, 1/16 *p*-value = 1.07E-04, 1/32 *p*-value = 5.29938E-05).

Fig. 3

a Bacterial growth (CFU/mL) determined using Miles–Misra–Irwin method for evaluating several dilutions of the original formulation of MSB and MSA medium, which were utilized as controls for these experiments. **b** Growth curves of *E. coli* WG5 (OD600) in several diluted MSB medium concentrations measured until reaching stationary phase. MSB medium was used as control for the experiment



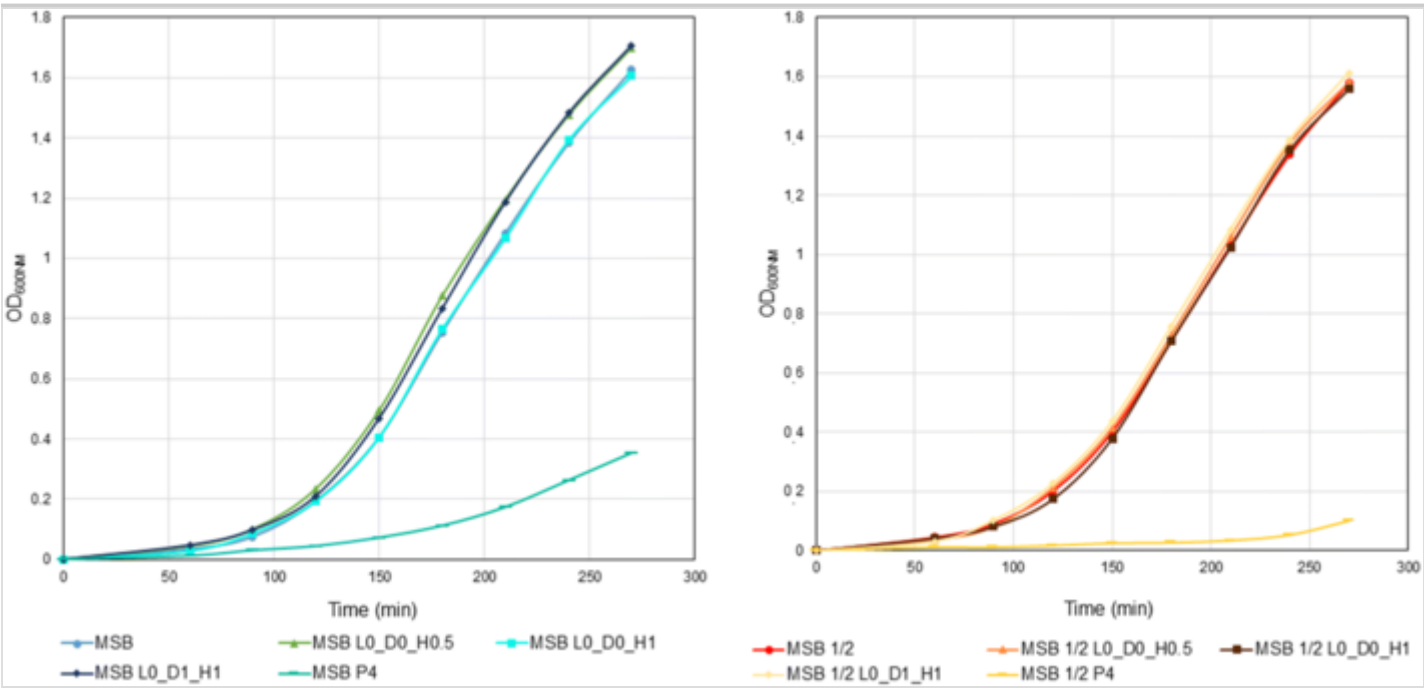
The comparison in Fig. 3b of different growth curves shows that there were no significant differences in growth for up to twofold dilutions (p-value = 0.949). However, threefold and fourfold dilutions, which were previously considered harmless for bacterial growth in our Miles–Misra–Irwin experiments, reduced the growth rate of *E. coli* cultures and were thus not considered for the following assays.

Growth Curve Analysis of Experimental Media

A global comparison of the growth curves of the remaining media was performed using MSB and twofold diluted MSB (MSB ½) as the base media. Concentrations and components added to media are the same as previously described in Table 2. Media L0_D0_H0.5 and L0_D1_H1 (Table 2) allowed a faster growth than the other media when combined with the original MSB (Fig. 4), however, not a significantly faster growth (p values = 0.838 and 0.862, respectively). As a consequence, the addition of sodium deoxycholate and disodium hydrogen phosphate dodecahydrate at the previously specified concentrations favored bacterial growth, reducing the time in which the culture reaches a cell density of > 10^9 CFU/mL (OD_{600} > 0.5). The significantly worst performances were clearly from both MSB and MSB ½ combined with P4 (p values = 0.031 and 0.012, respectively), resulting in a more than twofold slower growth rate compared to the controls. As shown in Fig. 4, there were no differences between the other potential media and the control used for both MSB and MSB ½.

Fig. 4

Representative growth curves of media with added components at determined optimal concentrations using MSB and MSB ½ as core media. (L0 = 0 g/L of sodium lauryl sulfate; D0 = 0 g/L of sodium deoxycholate; D1 = 0.1 g/L of sodium deoxycholate; H0.5 = 1.35 g/L of disodium hydrogen phosphate dodecahydrate; H1 = 2.7 g/L of disodium hydrogen phosphate dodecahydrate; P4 = 4.0 g/L of sodium propionate)

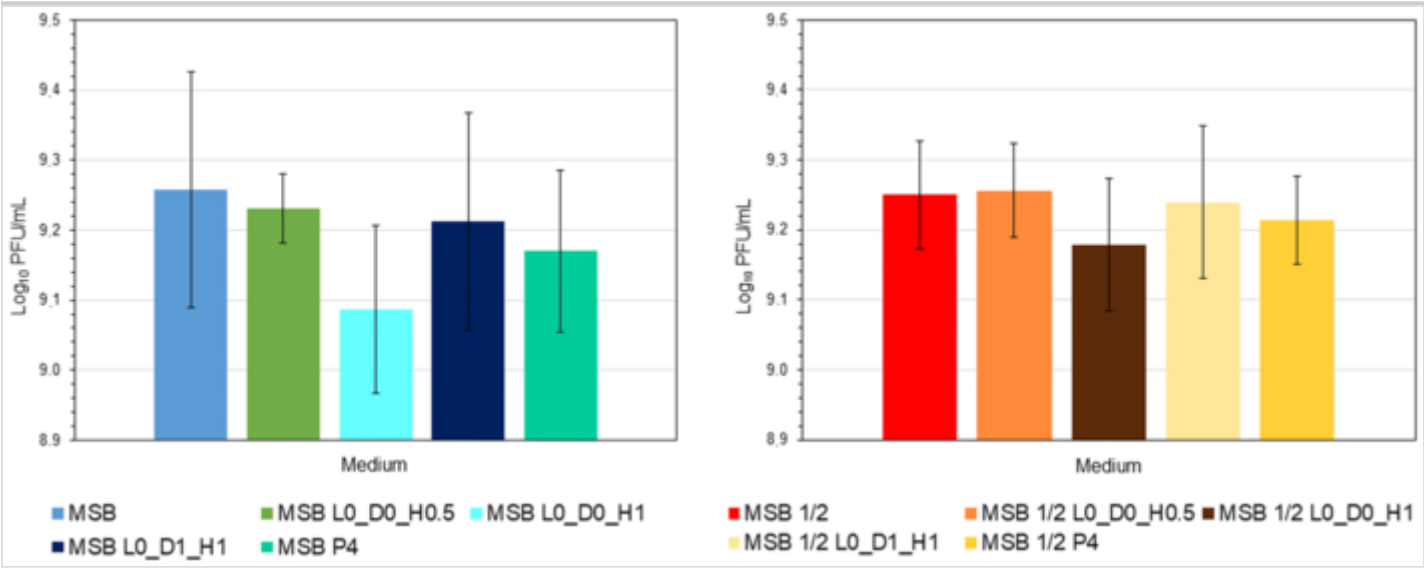


Plaque-Forming Unit Enumeration in the Experimental Media

PFU/mL was determined by DAL assays for all the experimental media based on MS media and MS ½ media, according to the results obtained in both the bacterial CFU/mL enumeration and growth curve measurement experiments. The extra components added to these media were identical to those added in the previous experiments (Table 2). No significant differences were observed in enumeration efficiency in terms of the components added to medium (Fig. 5) (*p*-value = 0,179). In relation to media reduction, twofold dilution yielded no significant changes in PFU/mL enumeration either (*p*-value = 0.714).

Fig. 5

Enumeration of somatic bacteriophages of the different studied combinations using the MSB, MSA, and MSAss media complete or ½ diluted with or without supplementation of the selected components. Selected components: L0 = 0 g/L of sodium lauryl sulfate; D0 = 0 g/L of sodium deoxycholate; D1 = 0.1 g/L of sodium deoxycholate; H0.5 = 1.35 g/L of disodium hydrogen phosphate dodecahydrate; H1 = 2.7 g/L of disodium hydrogen phosphate dodecahydrate; P4 = 4.0 g/L of sodium propionate; MSB = No potential components were added to the media



Cost Estimation

Using the ten newly designed media, a simple estimated cost analysis per sample was also performed, using the original MS media as a reference (MSB, MSA, or ssMSA). Estimations were made assuming the necessity of at least 10 plates of each media per sample, and the cost of each reagent was based on the commercial prices of the components used in our experiments. Results of this analysis are summarized in Table 3. The addition of new components in the medium could cause a maximum cost increase of 5%, in the case of sodium propionate. However, these slight increases might be mitigated by dilutions of the base media, which could result in a 27–33% reduction of analytical costs, depending on the additional components.

Table 3

Cost estimates per sample and fluctuation in comparison to original medium of the selected media

Medium	Cost of media per analyzed sample (€)	% of cost increase/decrease
MS	3.54	0.00
MS L0_D0_H0.5	3.57	0.78
MS L0_D0_H1	3.60	1.56
MS L0_D1_H1	3.62	2.21
MS P4*	3.74	5.80
MS 1/2	2.36	–33.38
MS 1/2 L0_D0_H0.5	2.39	–32.60
MS 1/2 L0_D0_H1	2.41	–31.82
MS 1/2 L0_D1_H1	2.42	–31.66
MS 1/2 P4	2.56	–27.57

*Best performing in terms of CFU/mL

Discussion

Out of the nine components tested, four had a significantly positive effect on bacterial growth as measured by CFU/mL enumeration: sodium laury sulfate, sodium deoxycholate, disodium hydrogen phosphate dodecahydrate, and sodium propionate. Although none of them had a significantly positive influence on bacteriophage detection by PFU/mL enumeration, the growth rate of the host strain was improved.

A possible positive synergy between sodium deoxycholate and disodium hydrogen phosphate dodecahydrate was found at 0.1 g/L and 2.7 g/L, respectively. Synergy was observed in both CFU/mL and OD measurements, while bacteriophage enumeration remained similar to the MSB control. A similar effect was observed with the addition of disodium hydrogen phosphate dodecahydrate at 1.35 g/L. The improvement in the bacterial growth rate induced by these combinations could indirectly benefit bacteriophage enumeration by decreasing the time of analysis.

The fourfold dilution of MSA did not improve the overall bacterial growth rate or therefore the enumeration of bacteriophages (PFU). However, the growth rate of the original MSB was maintained with twofold dilutions, thus confirming our initial hypothesis that the MS media contain an excessive amount of nutrients and could be diluted to save costs. Furthermore, this modification could also be applied for the analysis of larger sample volumes in enrichment cultures intended for absence/presence tests (Anonymous 2000), which currently use twofold concentrated media to analyze equal volumes of sample. As diluting the media would not significantly affect the measurements, 1× MSB could be used directly without reducing the growth of the host strain or the propagation of bacteriophages.

In conclusion, whereas the supplementation of MS media with new compounds rarely slowed bacterial growth, some combinations of compounds slightly improved the growth rate without altering bacteriophage enumeration. More studies are required to analyze the effects of these compounds and further optimize the quantity to be added to the media. In particular, the feasibility of their supplementation on a larger scale should be assessed from an economical point of view. Possible extra costs could nevertheless be offset by the dilution of the media, which can be done up to fourfold without significantly altering bacterial growth and up to twofold without altering either growth rate or bacteriophage enumeration. According to the estimates performed in this study, a 33% saving in costs could be achieved by reducing the MS media concentration by half. After the addition of different components, the savings were slightly lower, with a minimum of 27%. These results create new possibilities for designing improved tests or kits for bacteriophage detection, as any added costs per sample arising from the new components would be more than offset by the savings from media dilution.

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Author Contributions

All authors contributed to the study conception and design. ARB, JM and DTA designed the study, DTA performed the experiments, MM and ARB supervised the work, DTA wrote the manuscript and ARB and MM revised and approved the final version of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare the submitted work was not carried out in the presence of any personal, professional or financial relationships that could potentially be construed as a conflict of interest.

Research Involving Human Participants and or Animals This article does not contain any studies with human participants or animals performed by any of the authors.

References

Adams, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers.

Anonymous, (1995). *ISO 10705-1. Water quality: Detection and enumeration of bacteriophages—Part 1: Enumeration of F-specific RNA Bacteriophages*. Geneva: International Organization for Standardization.

Anonymous, (2000). *ISO 10705-2. Water quality: Detection and enumeration of bacteriophages—Part 2: Enumeration of somatic coliphages*. Geneva: International Organization for Standardization.

Anonymous, (2003a). *ISO 10705-3. Water quality: Detection and enumeration of bacteriophages—Part 3: Validation of methods for concentration of bacteriophages from water*. Geneva: International Organization for Standardization.

Anonymous, (2003b). *U.S. EPA: Results of the interlaboratory validation of EPA method 1601 for presence/absence of male specific (F+) and somatic coliphages in water by two-step enrichment. EPA 821-R-03-015*. Washington D.C.: Environmental Protection Agency.

Anonymous, (2003c). *U.S. EPA: Results of the interlaboratory validation of EPA method 1602 for enumeration of male specific (F+) and somatic coliphage in water by single agar layer (SAL). EPA 821-R-03-016*. Washington D.C.: Environmental Protection Agency.

Armon, R., & Kott, Y. (1996). Bacteriophages as indicators of pollution. *Critical Reviews in Environmental Science and Technology, 26*, 299–335.

Ashbolt, N. J., Grabow, O. K., & Snozii, M. (2001). Indicators of microbial water quality. In L. Fewtrell & J. Bartram (Eds.), *Water quality: Guidelines Standards and Health* (pp. 289–316). London: IWA Publishing.

Atlas, R. M. (2010). *Handbook of microbiological media*. Boca Raton, FL: CRC Press.

Borchardt, M. A., Haas, N. L., & Hunt, R. J. (2004). Vulnerability of drinking-water wells in la crosse, wisconsin, to enteric-virus contamination from surface water contributions. *Applied and Environmental Microbiology, 70*(10), 5937–5946.

Brion, G. M., Meschke, J. S., & Sobsey, M. D. (2002). F-specific RNA coliphages: Occurrence, types, and survival in natural waters. *Water Research, 36*(9), 2419–2425.

Christen, P., & Raimbault, M. (1991). Optimization of culture medium for aroma production by Ceratocystis fimbriata. *Biotechnology Letters, 13*, 521–526.

Davies, K. D. (1993). Design of experiments for predictive microbial modelling. *Journal of Industrial Microbiology & Biotechnology, 12*, 295–300.

Epa, U. S. (2016). *Coliphage experts workshop: Discussion topics and findings. Office of water. EPA 823-F-16F001*. Washington D.C.: Environmental Protection Agency.

Farnleitner, A., & A. Blanch. (2017). Microbial Indicators-"Workhorses" in the Field of Health-related Water Quality Testing. In Rose, J. B., & Jiménez-Cisneros J. B. (Eds.) Global Water Pathogen Project, eds. . https://www.waterpathogens.org (Farnleitner, A., & Blanch A. (Eds.) Part 2 Indicators and Microbial Source Tracking Markers). Michigan State University, E. Lansing, MI, UNESCO. https://www.waterpathogens.org/book/editorial

Feachem, R. G., Bradley, D. J., Garelick, H., & Mara, D. D. (1983). *Sanitation and disease: Health aspects of excreta and wastewater management*. Chichester, UK: Wiley.

Food and Drug Administration and Interstate Shellfish Sanitation Commission. (2015). *National sanitation program Guide for the Control of Molluscan Shellfish*. College Park, MD: Center for Food Safety and Applied Nutrition.

Francaise, R. (2014). *Arrêté du 25 juin 2014 modifiant l'arrêté du 2 août 2010 relatif à l'utilisation d'eaux issues du traitement d'épuration des eaux résiduaires urbaines pour l'irrigation de cultures ou d'espaces verts*. Paris: Gouvernement Français.

Gerba, C. P., Goyal, S. M., LaBelle, R. L., Cech, I., & Bodgan, G. F. (1979). Failure of indicator bacteria to reflect the occurrence of enteroviruses in marine waters. *American Journal of Public Health, 69*(11), 1116–1119.

Grabow, W. (2001). Bacteriophages: update on application as models for viruses in water. *Water SA, 27*(2), 251–268.

Havelaar, A. H., Furuse, K., & Hogeboom, W. M. (1986). Bacteriophages and indicator bacteria in human and animal faeces. *Journal of Applied Bacteriology, 60*(3), 255–262.

Hsu, F. C., Shieh, Y. S. C., & Sobsey, M. D. (2002). Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. *Journal of Food Protection, 65*(1), 93–99.

IAWPRC Study group on health related water microbiology. (1991). Bacteriophages as model viruses in water quality control. *Water Research, 25*, 529–545.

Jofre, J. (2007). Indicators of waterborne enteric viruses. In A. Bosch (Ed.), *Human viruses in water* (pp. 227–249). Amsterdam: Elsevier Science.

Jofre, J., Lucena, F., Blanch, A. R., & Muniesa, M. (2016). Coliphages as model organisms in the characterization and management of water resources. *Water, 8*(5), 199.

Keswick, B. H., Gerba, C. P., DuPont, H. L., & Rose, J. B. (1984). Detection of enteric viruses in treated drinking water. *Applied and Environmental Microbiology, 47*(6), 1290–1294.

Kott, Y., Roze, N., Sperber, S., & Betzer, N. (1974). Bacteriophages as viral pollution indicators. *Water Research, 8*(3), 165–171.

McFarland, J. (1907). Nephelometer: An instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association, 14*, 1176–1178.

Miles, A. A., Misra, S. S., & Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. *Journal of Hygiene, 38*(6), 732–749.

Mooijman, K. A., Ghameshlou, Z., Bahar, M., Jofre, J., & Havelaar, A. H. (2005). Enumeration of bacteriophages in water by different laboratories of the European Union in two interlaboratory comparison studies. *Journal of Virological Methods, 127*(1), 60–68.

Muniesa, M., & Jofre, J. (2004). Factors influencing the replication of somatic coliphages in the water environment. *Antonie van Leeuwenhoek, 86*(1), 65–76.

Nappier, S. P., Hong, T., Ichida, A., Goldstone, A., & Eftim, S. E. (2019). Occurrence of coliphage in raw wastewater and in ambient water: A meta-analysis. *Water Research, 153*, 263–273.

NHMRC & NRMMC. (2011). *Australian drinking water guidelines paper 6 national water quality management strategy*. Canberra: National Health and Medical Research Coucil, Australian Government.

North Carolina Administration. (2011). *North carolina administrative code 15A NCAC 2U*. Raleigh: North Carolina Administration.

Payment, P., Siemiatycki, J., Richardson, L., Renaud, G., Franco, E., & Prevost, M. (1997). A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. *International Journal of Environmental Research and Public Health, 7*(1), 5–31.

Plackett, R. L., & Burman, J. P. (1946). The design of optimum multifactorial experiments. *Biometrika, 33*(4), 305–325.

Queensland Government. (2005). *Queensland water recycling guidelines*. Brisbane: Queensland Government.

República de Colombia. (2014). *Decreto no 1287. Criterios para el uso de biosólidos generados en plantas de tratamiento de aguas residuales municipales*. Bogota: Republica de Colombia.

U.S. EPA. (2015). *Review of coliphages as possible indicators of fecal contamination for ambient water quality. EPA Office of Water 820-R-15-098*. Washington D.C.:

Environmental Protection Agency.

Western Australian Government. (2012). *Western Australia guidelines for biosolids management*. Perth: Western Australian Government.

WHO. (2017). *Potable reuse. Guidance for producing safe drinking-water*. Geneva: World Health Organization.

WHO. (2017). *Guidelines for drinking-water quality: Fourth edition incorporating the first addendum*. Geneva: World Health Organization.