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# The Effect of Various Carbonate Sources on the Survival of *Escherichia coli* in Dairy Cattle Manure

Ceri E. Arthurs,[1] Graeme N. Jarvis,[1] James B. Russell[1,2]

[1]Section of Microbiology, Wing Hall, Cornell University, Ithaca, NY 14853, USA
[2]Agricultural Research Service, USDA, Ithaca, NY 14853, USA

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**Abstract.** Manure slurries (n = 3) prepared from the feces and urine of lactating dairy cattle (1 part urine, 2.2 parts feces, and 6.8 parts distilled water) had an initial pH of 8.6 ± 0.1; dissolved carbonate concentrations of 48 ± 4 mM, and *Escherichia coli* counts of 5.9 ± 0.7 logs per ml slurry. The pH of untreated slurries declined to pH 7.0 ± 0.1 by the 10th day of incubation, and the *E. coli* count increased approximately 10-fold ($P < 0.05$). When slurries were treated with $Na_2CO_3$, $K_2CO_3$, $NaHCO_3$ or $Na_2CO_3 \cdot NaHCO_3$ (0 to 16 g/kg slurry), the dissolved carbonates increased in a linear fashion, but only $Na_2CO_3$ and $K_2CO_3$ (8 g/kg or greater) or $Na_2CO_3 \cdot NaHCO_3$ (16 g/kg) ensured an alkaline pH. Even relatively low concentrations of $Na_2CO_3$ or $K_2CO_3$ (8 or 12 g/kg) caused a decrease in *E. coli* viability ($P < 0.05$), and *E. coli* could not be detected if 16 g/kg was added (day 5 or 10 of incubation). $Na_2CO_3 \cdot NaHCO_3$ also caused a decrease in *E. coli* viability, ($P < 0.05$), but some *E. coli* (approximately $10^4$ cells per g) were detected on day 10 even if the concentration was 16 g/kg. $NaHCO_3$ did not prevent the decrease in pH or cause a decrease in *E. coli* numbers ($P > 0.05$). Calculations based on the Henderson-Hasselbalch equation (pH and dissolved carbonates) indicated that little *E. coli* killing was noted until the dissolved carbonate anion concentrations ($CO_3^{-2}$) were greater than 1 mM, but bicarbonate anion ($HCO_3^{-}$) concentrations as high as 180 mM did not affect *E. coli* viability. These results are consistent with the idea that carbonate anion has antimicrobial properties and can kill *E. coli* in dairy cattle manure.

Dairy cattle in the United States produce more than 100 billion kg of manure each year, and this manure is used as a fertilizer [5, 11]. It had generally been assumed that bacteria from dairy cattle manure would be trapped by the soil matrix [6], but these bacteria can migrate through soil into the ground water [2]. Because coliform bacteria and *Escherichia coli* O157:H7 can survive for long periods of time in stored cattle manure [7], there is a risk of ground water contamination [3].

Manure can be treated with chemicals to kill coliforms and other bacteria, but these methods are expensive or environmentally unsound [10]. Temperatures generated by anaerobic sewage digesters are often too low to destroy all the pathogens, and few anaerobic digesters have ever been installed on dairy farms [10]. Recent work indicates that *E. coli* in stored dairy cattle manure can be killed by a treatment that employs sodium carbonate and alkali, and the estimated treatment cost was less than $10 per cow per year [4].

The sodium carbonate and alkali treatment was ineffective if the pH was less than 8.5, but the nature of the pH dependency was not precisely defined [4]. When the pH becomes progressively more alkaline, the bicarbonate buffer system is shifted from bicarbonate anion, $HCO_3^{-1}$, to sesquicarbonate anion, $HCO_3^{-1}CO_3^{-2}$, and ultimately to carbonate anion, $CO_3^{-2}$. The following experiments were designed to

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*Correspondence to:* J.B. Russell at Cornell University; *email:* jbr8@cornell.edu

assess the effect of various carbonate sources on the survival of *E. coli* in stored dairy cattle manure.

## Materials and Methods

**Manure.** Fecal and urine samples were obtained from lactating dairy cattle (n = 12 and n = 4, respectively). Fecal samples were obtained directly from the rectum. Urine samples were collected from the vulva after tactile stimulation. The cattle were fed a typical lactation ration that consisted of 31% corn silage, 22% high moisture shell corn, 15% hay crop silage, 8% wheat middlings, 7% soybean meal, 5% cotton seed meal, 3% corn gluten feed, 3% distillers grains, 3% fish meal, and 3% mineral mix (dry matter basis). Fecal and urine samples were stored at 5°C until use.

**Artificial slurries.** The artificial slurries were formulated as 1 part urine, 2.2 parts feces, and 6.8 parts distilled water. The slurries were dispensed (80 ml, n = 3) into 120 ml sterile containers and were incubated aerobically at room temperature. The treatments were $Na_2CO_3$, $K_2CO_3$, $NaHCO_3$ or $Na_2CO_3{\cdot}NaHCO_3$ (0, 4, 8, 12, and 16 g/kg slurry). The slurries were sampled (6 ml) at 0, 5, 10, and 50 days.

**pH.** Each slurry was uncapped, mixed, and pH was measured directly with a combination electrode.

**Dissolved carbonates.** Slurry samples (5 ml) were placed in tubes (150 × 18 mm) that were sealed with butyl rubber stoppers and aluminum seals. Concentrated HCl (2 ml) was injected into each tube through the rubber stopper with a hypodermic syringe and needle. The tubes were mixed and left for 1 h at room temperature. The volume of carbon dioxide released from the slurry was measured as gas displacement by using a well lubricated hypodermic syringe (35 ml) and needle. Standard gas formula was used to express the carbon dioxide released as mmol carbonates ($CO_3^{2-}$, $HCO_3^-$ or $CO3^{-2}$) per liter of slurry (mM): $CO_2$ released (mM) = gas displacement (ml) ÷ 22.4 ml/mmol ÷ sample volume (5 ml) × 1000 ml/liter.

***E. coli* counts.** Slurry samples (5 ml) were placed in a tube and mixed vigorously with a vortex. Once the solid materials had settled, a supernatant sample (0.5 ml) was serially diluted (10-fold increments) in tubes (13 × 100 mm) that contained (4.5 ml) sodium lauryl sulfate broth (35.6 mg/ml). The tubes (n = 3) were incubated for 48 h at 37°C, and the highest dilutions showing growth were recorded. These were streaked on LB agar plates (10 g Trypticase, 5 g yeast extract, 5 g NaCl, and 2 g agar per liter). The presence of *E. coli* was confirmed by standard tests as indicated in the FDA manual [8]. Each bacterium was lactose positive, gas positive, methyl red positive, Voges-Proskauer negative, and citrate negative.

**Statistics.** All tests and enumerations were performed in triplicate, and significance ($P < 0.05$) was assessed with a Student's *t*-test [9].

## Results

When sodium carbonate was added to manure slurries, the dissolved bicarbonate/carbonate concentrations increased from 50 to 150 mM in a linear fashion (fig. 1a). The pH of untreated slurries decreased from 8.6 to 7.0, but sodium carbonate increased the final pH (Fig. 1b). When the sodium carbonate additions were greater than 8 g/kg, the final pH was always greater than 8.5. *E. coli* persisted in untreated manure slurries, and the count on

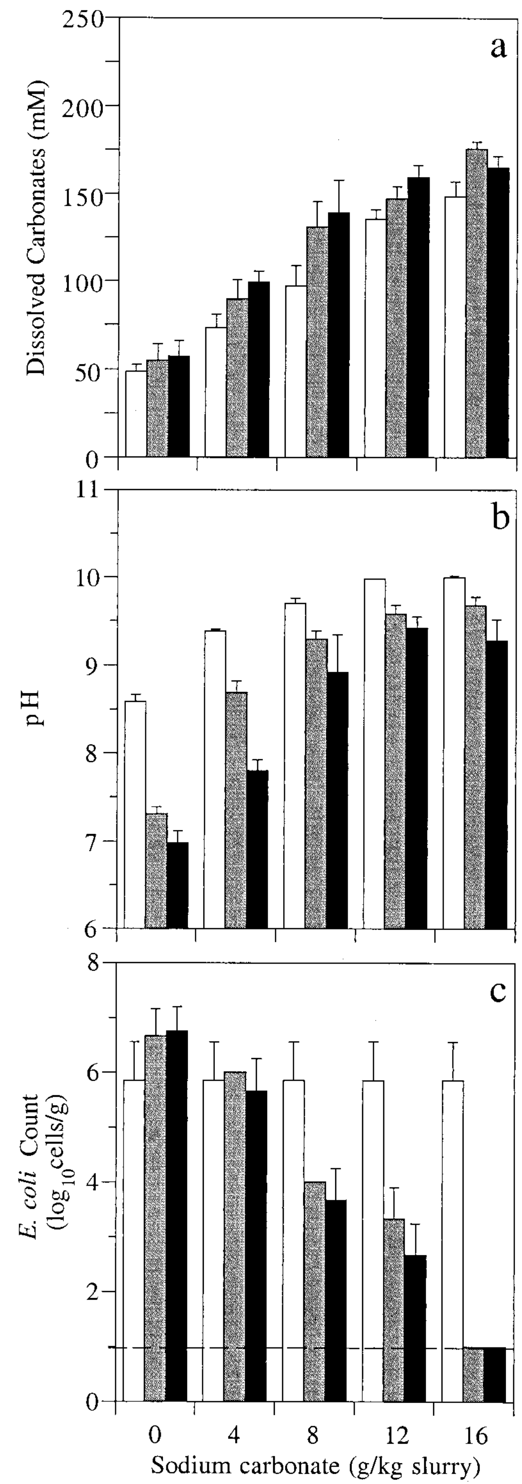


Fig. 1. The effect of sodium carbonate on dissolved carbonate concentrations (a), pH (b), and *E. coli* counts (c) of manure slurries in vitro. Open bars show day zero; grey bars show day 5; black bars show day 10. There were three replicates for each treatment (n = 3). The error bars are the standard deviations.

day 10 was as high as the count on day zero (Fig. 1c). Low concentrations of sodium carbonate had little effect on *E. coli* survival, but the count decreased if the concentrations were greater than or equal to 8 g/kg ($P < 0.05$). When the sodium carbonate addition was 16 g/kg, *E. coli* could no longer be detected on day 5 or 10. The *E. coli* counts on day 10 tended to be lower than counts on day 5, but this difference was not significant ($P > 0.05$). Potassium carbonate increased the dissolved carbonate concentration, increased the pH, and killed *E. coli* nearly as well as sodium carbonate ($P < 0.05$, data not shown).

Sesquicarbonate increased the dissolved bicarbonate/carbonate concentrations from 50 to 150 mM (Fig 2a), but it did not have as large an effect on final pH as sodium carbonate (Fig 2b). When the sesquicarbonate concentration was 8 g/kg, the pH on day 5 was 8.5, but the pH declined to 7.5 by day 10. Only high concentrations of sesquicarbonate (12 and 16 g/kg) were able to ensure a final pH of 8.5 on day 10. High concentrations of sesquicarbonate killed some of the *E. coli* ($P < 0.05$), but the final count was approximately $10^4$ viable cells per g slurry on day 10 (Fig. 2c). Sodium bicarbonate also increased the dissolved bicarbonate/carbonate concentrations (Fig. 3a), but it had little effect ($P > 0.05$) on final pH (Fig. 3b) or the viable *E. coli* count (Fig. 3c).

By using the $pK_a$ values of carbonic acid (6.35 and 10.33), the dissolved bicarbonate/carbonate concentrations, final pH, and the Henderson-Hasselbalch equation, it was possible to calculate the dissolved carbonate anion ($CO_3^{-2}$) concentrations. When the dissolved carbonate anion concentration was less than 1 mM, little *E. coli* killing was observed (Fig. 4). However, when the dissolved carbonate anion concentrations increased from 1 to 30 mM, there was a dramatic decrease in *E. coli* viability ($P < 0.05$).

## Discussion

In its strictest sense, manure is a mixture of feces and urine, and dairy cattle produce approximately 2.2 times as much feces as urine [5]. However, manure from modern dairies is often stored outside in ponds or lagoons, and the stored manure can be diluted by rainwater. In some operations, the wash water from the milking parlor is also added. Dougherty et al. [5] indicated that the dry matter content of modern dairy cattle manure ranges from 2% to 5% (wt to wt), and our manure slurries had a dry matter content of 3%.

Previous workers indicated that coliform bacteria could survive for long periods of time in dairy cattle manure [7]. The coliform count of manure slurries increased approximately 1 log over the first days of incu-

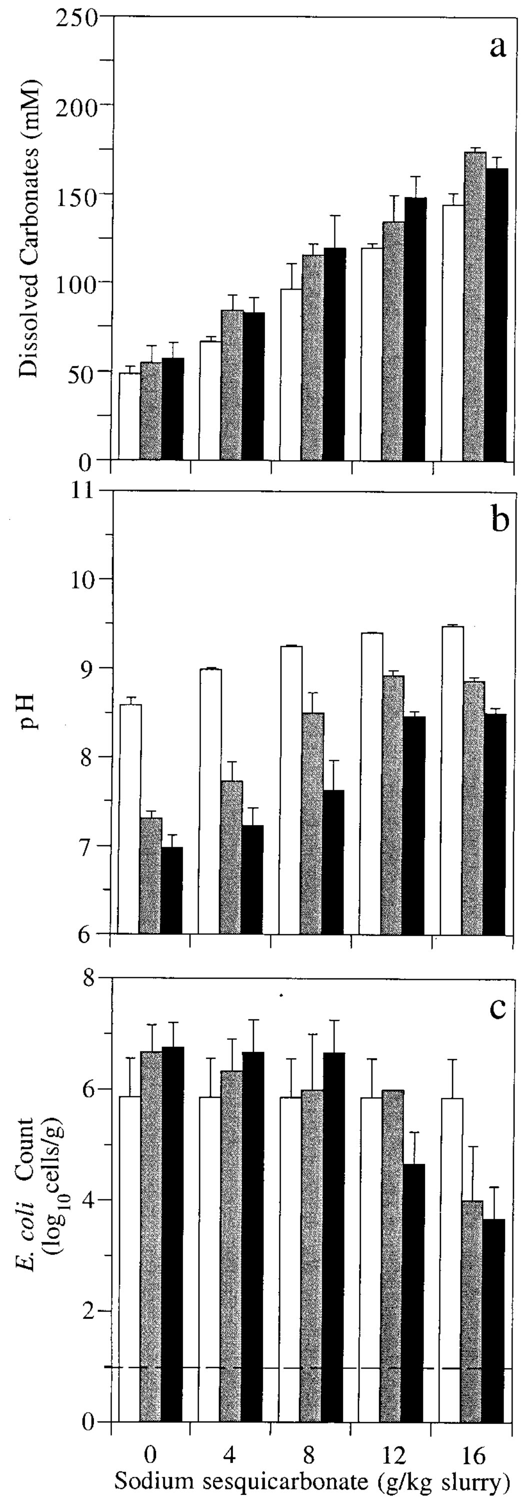


Fig. 2. The effect of sodium sesquicarbonate on dissolved carbonate concentrations (a), pH (b), and *E. coli* counts (c) of manure slurries in vitro. Open bars show day zero; grey bars show day 5; black bars show day 10. There were three replicates for each treatment (n = 3). The error bars are the standard deviations.

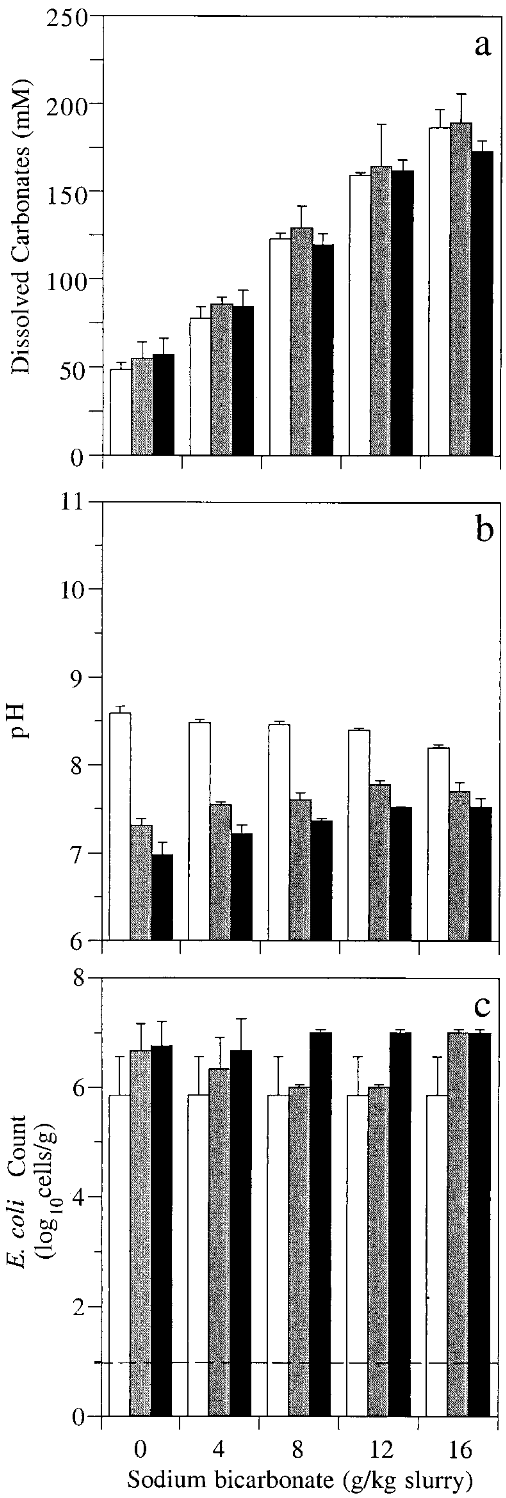


Fig. 3. The effect of sodium bicarbonate on dissolved carbonate concentrations (a), pH (b), and *E. coli* counts (c) of manure slurries in vitro. Open bars show day zero; grey bars show day 5; black bars show day 10. There were three replicates for each treatment (n = 3). The error bars are the standard deviations.

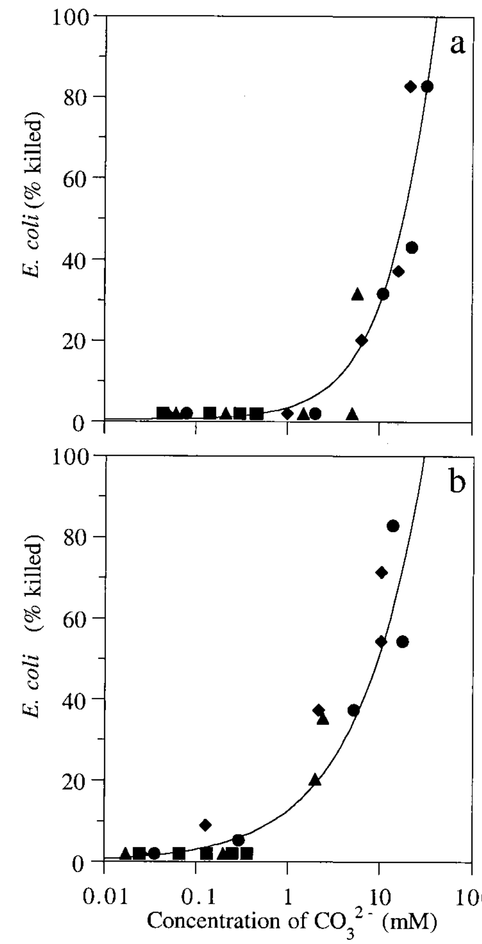


Fig. 4. The effect of carbonate anion ($CO_3^{-2}$) concentration on the percentage of *E. coli* that were killed by each treatment (●, sodium carbonate; ◆, potassium carbonate; ▲, sodium sesquicarbonate; ■, sodium bicarbonate) on day 5 (part a) or day 10 (part b). Each point is the mean of 3 determinations.

bation at room temperature (Figs. 1–3). The coliform count eventually declined, but untreated slurries on day 50 still had approximately $10^5$ viable cells per g slurry (data not shown). Additional tests (brilliant green, indole production, citrate utilization, Voges Proskauer test, methyl red test, and growth on MacConkey's agar) indicated that the highest dilutions showing visible growth always contained *E. coli*.

Corral et al. [1] noted that *E. coli* could be killed by a "sodium bicarbonate" treatment, but significant killing was not observed until the pH was made alkaline with NaOH (pH 8.6). When the pH becomes alkaline, bicarbonate anion undergoes an additional dissociation, and carbonate anion concentrations increase. If the pH is 8.6, carbonate anion accounts for only 2% of the dissolved carbonates, but this value increases as a logarithmic function of pH.

Previous work indicated that the sodium carbonate treatments could eliminate *E. coli* from dairy cattle manure only if the pH was alkaline, and sodium hydroxide was added to make sure that the pH was always greater than 8.5 [4]. These results indicated that pH was indeed a critical aspect of the antibacterial activity, but the total carbonate concentration and pH were not varied in a systematic fashion. Sodium carbonate can act as a base to increase pH significantly, but sodium bicarbonate solutions are never strongly alkaline.

Calculations based on the Henderson-Hasselbalch equation, dissolved bicarbonate/carbonate concentrations, and final pH indicated bicarbonate anion concentrations increased as sodium carbonate was added, but the change was only threefold (50 to 150 mM). Over the same range of sodium carbonate additions, the dissolved carbonate anion concentrations increased approximately 3000-fold (0.01 to 30 mM; Fig. 4). Because little *E. coli* killing was noted until the dissolved carbonate anion was greater than 1 mM, it appeared that carbonate anion rather than bicarbonate anion was the antibacterial agent.

Bicarbonate is a relatively nonreactive monovalent anion, but carbonate anion forms insoluble complexes with divalent cations ($Mg^{++}$, $Ca^{++}$, $Zn^{++}$, and $Fe^{++}$) that play an important role in bacterial physiology [12]. Previous work indicated that the carbonate and alkali treatment of cattle manure killed virtually all *E. coli* in only 5 days [4]. Because carbonate, bicarbonate, and sesquicarbonate treatments that produced less than 1 mM carbonate anion did not kill the *E. coli* even if the incubation period was 50 days (data not shown), it appears that carbonate stoichiometry is a more important variable than time per se.

These results support the idea that carbonate can be used as an antimicrobial to kill *E. coli* in stored dairy cattle manure, but pH is an important feature of this effect. Sodium bicarbonate alone did not increase the pH or dissolved carbonate anion concentration significantly, and it was unable to kill *E. coli*. Because sodium carbonate and sodium hydroxide are relatively inexpensive chemicals, the cost of the manure treatment would be relatively low (<$10 per dairy cow per year). If sodium accumulation in soil is a concern, potassium carbonate and potassium hydroxide could be substituted, but these chemicals would increase the cost (approximately twofold).

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## Literature Cited

1. Corral LG, Post LS, Montville TJ (1988) Antimicrobial activity of sodium bicarbonate. J Food Sci 53:981–982
2. Craun GF (1991) Causes of waterborne outbreaks in the United States. Water Sci Technol 24:17–20
3. Deutrich V, Pioch G (1991) Risk of infection to man and animals by cattle slurry stored over years. Monatsh Vetmed 46:651–655
4. Diez-Gonzalez F, Jarvis GN, Adamovich DA, Russell JB (2000) Use of carbonate and alkali to eliminate *Escherichia coli* from dairy cattle manure. Environ Sci Technol 34:1275–1279
5. Dougherty M, Geohring LD, Wright P (1998) *Liquid manure application systems design manual*. Ithaca, New York: Northeast Regional Agricultural Engineering Services (NRAES)
6. Edmonds RL (1976) Survival of coliform bacteria in sewage sludge applied to a forest clearcut and potential movement into groundwater. Appl Environ Microbiol 32:537–546
7. Himathongkham S, Bahari S, Riemann H, Cliver D (1999) Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiol Lett 178: 251–257
8. Hitchins AD, Feng P, Watkins WD, Rippey SR, Chandler LA (1995) Food Drug Administration bacteriological analytical manual, 8th ed. Gaithersburg, MD: AOAC International Chap. 4
9. Sokal RR, Rohlf FJ (1969) Biometry. San Francisco: Freeman Co.
10. Strauch D, Ballarini G (1994) Hygenic aspects of the production and agricultural use of animal wastes. J Vet Med B 41:176–228
11. USDA-ERS-NASS (1999) *Milk cows and production—final estimates 1993–1997*; NASS Bulletin SB952. Washington, DC. National Agricultural Statistics Service
12. White D (1995) The physiology and biochemistry of prokaryotes. Oxford: Oxford University Press