






## A Need to Explain Apparent ‘Hard-to-biodegrade’ Cellulose Remainders in CO<sub>2</sub>-based Test Results

Martin A. Hubbe <sup>a,\*</sup>, Soojin Kwon <sup>b</sup>, Jesse S. Daystar <sup>c</sup>, Joel J. Pawlak <sup>a</sup> and Richard A. Venditti <sup>a</sup>

The extent of cellulose fiber biodegradation, according to many published studies, tends to reach a plateau value well below 100%. This editorial proposes that the apparent residue of not-biodegraded cellulose may be due to simplifying assumptions in a commonly used assay to quantify cellulose biodegradation. Some such tests are based on the production of CO<sub>2</sub>. The evolved CO<sub>2</sub> is removed from the air by an alkaline trap, which triggers a quantified addition of O<sub>2</sub> gas. However, N<sub>2</sub> and NH<sub>3</sub> gases are evolved during biodegradation. The theoretical amount of nitrogen might explain a shift of up to 0.63% in the extent of biodegradation. Another possibility is that the evolution of nitrogen-based gases starves the biological system of nitrogen, thereby terminating biodegradation in the test container. This editorial asks: “Is biodegradation of cellulose usually more complete in comparison to the results from standard tests?” If yes, that would match the fact that cellulose does not build up endlessly in the environment. These findings have direct implications for natural fibers such as cotton, which might be systematically underdetermined in standard biodegradation comparisons against synthetic fibers.

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Contact information: a: Department of Forest Biomaterials, College of Natural Resources, North Carolina State University, Campus Box 8005, Raleigh, NC, 27606; b: Gyeongsang National University, Department of Environmental Materials Science, College of Ag. & Life Sci., South Korea; c: Cotton Incorporated, 6300 Weston Parkway, Cary, NC 27516, US; Corresponding author: hubbe@ncsu.edu

### An Apparent Remainder of ‘Hard-to-biodegrade’ Cellulose

The generation of CO<sub>2</sub> in the course of cellulose biodegradation has been used to track and quantify the process (ISO 14851). One starts by weighing a specimen of known composition and calculating the amount of carbon dioxide that would theoretically be released following total hydrolysis of the polysaccharide content, which is assumed to be catalyzed by enzymes. A recent review article considered such studies (Hubbe *et al.* 2025). A remarkable finding of such studies is an apparent persistence of a remainder of about 10 to 40% of the cellulose at the end of the test period, based on the weighing and the calculations. The cellulose biodegradation appears to gradually slow down and then almost stop at about 60 to 90%, in many cases (Kwon *et al.* 2021; Smith *et al.* 2024).

There are practical reasons to expect biodegradation to continue steadily or even to accelerate in the later stages under lab conditions. First, whatever fungi or bacteria had been providing the enzymes to promote hydrolysis of the cellulose would have had time to build up, partly in response to the presence of the cellulose itself. The remaining cellulose can be expected to be substantially broken down and to become increasingly accessible to enzymatic attack, having already lost much of its substance. The remaining cellulose is expected to have a somewhat porous structure, due to the preceding enzyme action.

## Initial Attempts to Explain the “Hard to Break Down” Cellulose

Table 1 contains several initial explanations that were considered by the authors to account for the reported results. Each explanation is paired with some reasons that the authors were not satisfied with those explanations:

**Table 1.** Initially Considered Explanations for the Apparent Remainder of Unbiodegraded Cellulose

Hypothesized explanation	Reasons to doubt the explanation
The remainder of cellulose is too crystalline to be broken down readily.	Natural cellulose has a high crystallinity, much higher than the typical reported amount of remainder.
The tests should have been extended to longer times.	This is valid for some of the test conditions and plots. But many of the tests showed a horizontal plateau.
The calculation was inaccurate, perhaps due to neglect of moisture content of the specimen.	Analytical grade microcrystalline cellulose (MCC), usually used as a benchmark, is quite pure cellulose and low in moisture content.
The remaining cellulose is protected by a layer of lignin.	That concept cannot explain results found for cotton and for microcrystalline cellulose, <i>etc.</i>
Nutrients in the aqueous medium have become depleted.	The nutrients are expected to participate in a cycle of life, rather than getting used up.
Inhibiting substances build up in the enclosed environment to an extent that would not be found in nature.	Cellobiohydrolase is expected to be continually breaking down cellobiose, which is the most important expected inhibiting species.
The remaining cellulose becomes covered by a layer of denatured enzymes, blocking other enzymes.	Such proteinaceous material would be expected eventually to be broken down by proteinase enzymes.
Some CO <sub>2</sub> that evolves manages to pass through the alkaline solution trap without becoming neutralized.	The titration system, including the trap, can be calibrated with known amounts of CO <sub>2</sub> gas.
Some CO <sub>2</sub> that evolves leaks out.	The equipment is designed to avoid such leaks.

## Proposed Explanation Based on N<sub>2</sub> Evolution

Biodegradation cannot occur in the absence of proteins, which are a necessary component of both biological cells and enzymes. The biodegradation of proteins can be expected to result in the evolution of a combination of nitrogen gas (Jiang *et al.* 2026), the oxides of nitrogen (N<sub>2</sub>O and NO) (Jiang *et al.* 2026), and ammonia (Kappaun *et al.* 2018), each of which can be released to the gas phase. The test protocol ISO 14851-2019 that is often used to quantify biodegradation calls for the addition of ammonium chloride as part of the test medium. As a first step, a “Solution A” is prepared that contains 0.5 g of NH<sub>4</sub>Cl per liter, along with some other electrolytes. Each specimen is tested with the addition of 10 mL of Solution A per liter. In the work of Kwon *et al.* (2021), each specimen suspension was 400 mL. This implies 2 mg of NH<sub>4</sub>Cl per specimen. If 100% of the NH<sub>4</sub>Cl ended up as N<sub>2</sub>, that would amount to about 18.7 μmol of nitrogen gas. Kwon *et al.* (2021) added 80 mg of cellulose per test (with 400 mL of suspension). If all of that cellulose were biodegraded with the production of carbon dioxide gas, one can calculate that there would be 2960 μmol of CO<sub>2</sub> gas. The ratio of theoretical N<sub>2</sub> gas volume to CO<sub>2</sub> gas volume implies a maximum error (lower biodegradation than actual) by about 0.63%.

## Proposed Explanation Based on NH<sub>3</sub> from Degradation of Proteins

Biodegradation of protein results in some production of NH<sub>3</sub> by a urease pathway (Kappaun *et al.* 2018). Biodegradation of proteins also gives rise to CO<sub>2</sub> (Pols *et al.* 2020).

The ratio between release of NH<sub>3</sub> and CO<sub>2</sub> will depend on details of protein molecular structure, reaction pathways, and reaction kinetics. Arginine, a building block of proteins, is known to break down to urea, which then yields NH<sub>3</sub> (Wu 1995), with an expected ratio of NH<sub>3</sub> to CO<sub>2</sub> of 2:1 (Pols *et al.* 2020). To estimate the theoretical maximum amount of NH<sub>3</sub> gas, it will again be assumed that 100% of the NH<sub>3</sub> content will come from the NH<sub>4</sub>Cl. The calculation is the same as when considering N<sub>2</sub> evolution, above, except for a factor of two. Each mole of N atoms can result in one mole of NH<sub>3</sub> but only half a mole of N<sub>2</sub>. Thus, the theoretical maximum NH<sub>3</sub> production can account for about 1.26%, as an estimate for the erroneous lack of biodegradability of cellulose during a standard test.

### **Proposed Explanation Based on Active Nitrogen Depletion**

The action of enzymes, upon which biodegradation occurs, can be regarded as a catalytic process. A relatively small amount of proteinaceous material can theoretically break down a much larger amount of cellulose by operating multiple times. However, as was just shown, there exists a mechanism by which the system might become starved of the active nitrogen that is required for all biological processes. The biochemical pathways by which active nitrogen can be lost can be complex and hard to predict (Jiang *et al.* 2026).

### **Proposed Explanation Based on Solubility of CO<sub>2</sub> in Water**

At room temperature, the amount of CO<sub>2</sub> gas that will dissolve in water from the atmosphere is about 1.5 g/L. Assuming the same 400 mL of suspension, that would imply 0.6 g, which is equivalent to 13600 μmol. Although this value is about 4.6 times that of the CO<sub>2</sub> expected from the biodegradation of cellulose, there are several uncertainties to consider. The standard test employs an alkaline trap to remove CO<sub>2</sub> from the air space. Assuming complete equilibration, all of the CO<sub>2</sub> should eventually pass into the alkaline trap. However, it is not certain how much time would be required for such exchange. Hypothetically, the equilibration might be slowed down by a monolayer at the water surface, *e.g.* fatty acids from some cellulosic materials.

### **Proposed Explanations Based on Dynamics of Microbiology**

Pfeiffer and Bonhoeffer (2004) showed that when microbes are cultured under isolated conditions, analogously to the conditions of ISO 14851, there can be a shift of dominance in the microbial community. A second microbe may employ different enzymes that act on degradation products from the first. As a result of such changes, the production of cellulase might stop, or the cellulase already produced might become denatured. As microorganisms degrade biomass, they consume materials and grow in numbers. This additional biomass is not necessarily accounted for in biodegradation tests. The carbon content of bacteria has been reported to be ~50% by weight (Fagerbakke *et al.* 1996). Thus, a simple mass balance shows that a significant portion of the cellulose (*e.g.* 10% to 50%) could be converted into biomass carbon.

### **Summation**

Many questions remain. Is there evidence of depletion of active nitrogen when conducting standard tests of biodegradation? Do conditions allow suitably fast equilibration of CO<sub>2</sub> among the aqueous, gas, and alkaline trap phases? Does N<sub>2</sub> or NH<sub>3</sub> build up in the air space, thus displacing some of the intended flow of oxygen during the test? Are microbial population changes large enough to explain plateau effects? At least

some of these questions could be addressed by future experimentation. The authors hope that his editorial helps to draw more research attention to these issues. Among the explanations considered, the hypothesis based on denitrification and starvation of the protein-based enzyme system can explain not only the plateauing of biodegradation levels and the magnitude of the effects, but also the fact that such plateaus can be at widely different levels of apparent degradation. For commercially significant natural fibers such as cotton, resolving these measurement questions would confirm what field observation already suggests: that cellulose biodegrades more completely than standard tests indicate, providing a meaningful contrast to synthetic fibers that persist in the environment.

### References Cited

- Fagerbakke, K. M., Heldal, M., and Norland, S. (1996). "Content of carbon, nitrogen, oxygen, sulfur, and phosphorus in native aquatic and cultured bacteria," *Aquatic Microb. Ecol.* 10, 15-27. <https://doi.org/10.3354/ame010015>
- Hubbe, M. A., Daystar, J. S., Venditti, R. A., Pawlak, J. J., Zambrano, M. C., Barlaz, M., Ankeny, M., and Pires, S. (2025). "Biodegradability of cellulose fibers, films, and particles: A Review," *BioResources* 20(1), 2391-2458. <https://doi.org/10.15376/biores.20.1.Hubbe>
- ISO 14851 (2019). "Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by measuring the oxygen demand in a closed respirometer"
- Jiang, B. Q., Chen, X. F., Tian, Y. L., Yang, S. X., Tong, Y. B., and Niu, J. F. (2026). "Application of stable isotopes in revealing mechanisms of pollutants biodegradation in wastewater treatment: A review," *J. Environ. Chem. Eng.* 14(2), article 121250. <https://doi.org/10.1016/j.jece.2026.121250>
- Kappaun, K., Piovesan, A. R., Carlini, C. R., and Ligabue-Braun, R. (2018). "Ureases: Historical aspects, catalytic, and non-catalytic properties – A review," *J. Advan. Res.* 13, 3-17. <https://doi.org/10.1016/j.jare.2018.05.010>
- Kwon, S., Zambrano, M. C., Pawlak, J. J., and Venditti, R. A. (2021). "Effect of ligno-cellulosic fiber composition on the aquatic biodegradation of wood pulps and the isolated cellulose, hemicellulose and lignin components: Kinetic modelling of the biodegradation process," *Cellulose* 28(5), 2863-2877. <https://doi.org/10.1007/s10570-021-03680-6>
- Pfeiffer, T., and Bonhoeffer, S. (2004). "Evolution of cross-feeding in microbial populations," *Amer. Naturalist* 163(6), E126-E135. <https://doi.org/10.1086/383593>
- Pols, T., Singh, S., Deelman-Driessen, C., Gaastra, B. F., and Poolman, B. (2020). "Enzymology of the pathway for ATP production by arginine breakdown," *Febs J.* 288(1), 293-309. <https://doi.org/10.1111/febs.15337>
- Smith, M. M., Zambrano, M., Ankeny, M., Daystar, J., Pires, S., Pawlak, J., and Venditti, R. A. (2024). "Aquatic aerobic biodegradation of commonly flushed materials in aerobic wastewater treatment plant solids, seawater, and lakewater," *BioResources* 19(1), 1150-1164. <https://doi.org/10.15376/biores.19.1.1150-1164>
- Wu, G. Y. (1995). "Urea synthesis in enterocytes of developing pigs," *Biochem. J.* 312, 717-723. <https://doi.org/10.1042/bj3120717>