Beta-Glucosidase (BG) Test Information Rev. 1.0 Ward Laboratories Inc.

Introduction

Energy is harnessed in a plant through photosynthesis, a process by which carbon dioxide (CO_2) and water (H_2O), with the help of energy from the sun, is converted to different carbohydrates and oxygen (O_2). Plants store and use the various forms of carbohydrates, such as sugars, starches and cellulose, for maintenance, cell structure and growth. In general, carbohydrates are compounds that contain different ratios of carbon and water molecules that serve as a fundamental source of energy and that emphasizes the importance of the carbon cycle in the soil. In addition, plants can release carbohydrates from the roots to help cultivate distinct soil microbial communities, which use the carbohydrates as a main source of chemical energy.

When a plant dies, soil microbiology begins decomposing the strong, rigid structure of cellulose, the basic structural component of plant cell walls and fiber. Soil microbes produce a collective group of soil enzymes, known as cellulases, to disassemble the rigid structure of cellulose to release glucose molecules, a simple readily available sugar and important source of easily accessible energy for soil microbes. The cellulose structure is fractured by three dominant groups of enzymes: endoglucanases, exoglucanases and β -glucosidases (See Figure 1). Endoglucanases randomly cleave the long-chained cellulose into smaller fractions while exoglucanases further reduce the cellulose chain to cellulobiose units (two linked glucose molecules). Finally, β -glucosidases cleave the cellulobiose molecules to glucose, a readily available energy source for plant and microbial uptake.



Figure 1: Simplified schematic of cellulase enzymes acting on cellulose

The long-chained cellulose is fractured by the accumulated effort of exoglucanases, endoglucanases and βglucosidase enzymes to release glucose in the soil environment. (Image from Akhtar 2016)

4007 Cherry Ave. Kearney, NE 68847 www.wardlab.com 308-234-2418

Ward Laboratories, Inc.

Soil organic matter (SOM) serves as an important habitat for soil organisms and is a source of short- and long-term carbon storage. Increasing SOM provides numerous benefits to the soil such as improved soil structure and water retention. Traditional SOM measurements are based on a percentage of the whole soil system, making small, subtle changes in SOM due to changes in soil managements difficult to detect. Soil organic carbon (SOC) represents carbon stored in soil organic matter and the quantity and quality of SOC can indicate the availability of energy and nutrients for plants and soil microbes. Because of the pivotal role β -glucosidase (BG) plays in residue decompositions and the carbon cycle, the activity of BG is closely monitored to detect changes in the carbon cycle and SOC cycling in a shorter time span (approximately 2 years compared to 5 - 7 years through traditional laboratory methods). Greater soil microbial biomass often has higher BG activity indicating a soil microbial community's ability to break down plant residues and cycle nutrients through the soil, an important step to ensure nutrients are available in the soil for future crops. Thus, BG can provide an early indication of changes in SOC sooner than traditional total and organic C analysis can discern differences. Absence or suppression of BG prevents or reduces the cycling of soil nutrients that can impact plant health and alert producers to early issues in soil health.

Soil enzyme testing at Ward Laboratories is conducted by analyzing the consumption of a substrate and the release of a colored product. The consumption of product is measured over time and results are expressed as a rate of enzyme activity. By using controlled soil conditions (e.g. pH, temperature), the enzyme activity rates can indicate the potential activity for the soil enzymes under ideal conditions. This allows a comparison of potential enzyme activities between different soil management practices. Similarly, the same site can be tracked over time to monitor subtle changes in microbial dynamics and provide an indication of the microbial community response to changing environmental conditions and management.

Procedure Outline

Soil samples received by the laboratory should be cooled and in field moist condition. Each soil sample is passed through a 2 mm sieve and weighed into two centrifuge tubes $(1.00 \pm 0.05 \text{ g} \text{ each})$. The first subsample is referred to as the treatment sample and the second subsample is referred to as the control sample. Each vial receives 4 mL buffer solution. The treatment vial receives 1 mL substrate prior to all samples being incubated for 1 h at 37°C. After incubation, the control vial receives 1 mL substrate. All vials receive 4 mL stop buffer and 2 mL flocculant. Vials are centrifuged, filtered with Whatman 2V filter paper and analyzed on a spectrophotometer at 405 nm. Enzyme activity is expressed as ppm *p*-nitrophenol g⁻¹ dry weight soil h⁻¹.

Interpretation of soil enzyme activity requires an understanding of nutrient or organic matter cycling. Often, healthy, active systems have increased enzyme activity, relating to better cycling

4007 Cherry Ave. Kearney, NE 68847 www.wardlab.com 308-234-2418

Ward Laboratories, Inc.

of nutrients and organic matter quality in the soil. Nevertheless, sites with recent disturbances may have higher activity levels due to increased substrate availability when compared to nondisturbed sites. For example, a conventional tillage field versus a no-till field in its first year of transition may indicate the tilled field has higher enzyme activity. This is because the act of tilling a field provides aeration and better distribution of substrates to microbes. The increased activity cannot be fully sustained in this system and often causes the enzymes to access the nutrients in organic matter, leading to a loss of organic matter the following year. A list of common soil characteristics and soil management impacts can also be found in the interpretation guide.

Additional information will be added to the website as new information becomes available. Any questions regarding soil enzyme testing may be directed to biotesting@wardlab.com.

4007 Cherry Ave. Kearney, NE 68847 www.wardlab.com 308-234-2418