MONITORING THE HYDROLYTIC AND ENZYMATIC DEGRADATION OF BIOCOMPOSITES WITH A LUMINOTECH

N. Pons[1], F. Fesquet[1], N.Vidal[2]

[1]*MATERIAU INGENIERIE Sarl, Vallon de Fontanes, 2 rue des Acacias, 30520 Saint-Martin-de-Valgalgues, France*

[2]*YELEN Sarl, 10 boulevard Tempête, 13820, Ensues-la-Redonne*

A. INTRODUCTION

In order to overcome the problem of reproducibility inherent in biodegradation tests and reduce testing time, Matériau Ingénierie and **Yelen*** have developed a biodegradation test by chemiluminescence. The approach adopted is molecular to monitor in real time the solubilization of enzymatic attack markers. These markers, react with one or more reaction intermediates in order to produce photons by luminescence (dedicated reagent). For the PLA, the marker is lactic acid (or lactate) from the action of hydrolytic enzymes (*Figure 1*). The number of photons emitted is measured using a Luminotech.

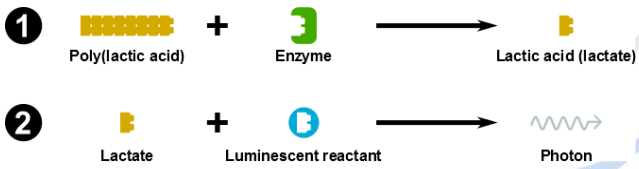

Figure 1: Method Principle (case of the enzymatic degradation of PLA).

The use of enzyme facilitates the analysis of biodegradability in comparison with microbial methods because enzymes are catalysts for reactions and continuing to act as the temperature and pH are optima. Instead of selecting a specific enzyme, our method is based on the use of "enzyme cocktail" and has the advantage of being less expensive while being biomimetics.

The method allows control at the molecular level the water resistance of biodegradable materials in terms of use.

The results presented in this article were obtained on samples of poly (lactic acid) (or PLA) reinforced with glass fibers. For the needs of this study, the method presented above was expanded to monitor other two reactions:

- chemical hydrolysis of PLA (kinetics of degradation by water without enzyme);
- Chemical hydrolysis of the glass, some formulations having the ability to be altered by water (the degradation markers are silicates).

B. MATERIALS & METHODS

1) *Materials & Processing*

The samples are from specimens obtained by injection. PLA is a PLA 7000D® (NatureWorks). The introduction of fibers into the PLA was performed by extrusion. Different types of fibers were used :

- a hemp fiber [PLA-NF], cut into short fibers (length of 5mm before extrusion);
- a commercial glass fiber [PLA-CF];
- and two glass fiber alterable by water of various formulations [PLA-AF_1 and PLA-AF_2].

The weight fraction of PLA biocomposites is 70%. The three glass fibers have a diameter of 13µm and a length of 5mm before extrusion.

2) *Degradation conditions*

Degradation is made in test tubes on samples in powder form (Ø 1.5-2 mm) obtained by grinding in a knife mill.

The powder concentration in the aggressive medium is 250 mg/ml. Three test conditions were tested:

- an abiotic medium (pure water, 37°C/48 h);
- an abiotic medium (pure water, 65°C/24 h);
- a biotic medium (water + enzyme cocktail, 37°C/48h).

The conditioning temperature control is carried out using a thermostatic bath.

The preparation of the biotic medium was performed using a kit developed by Yelen.

3) *Photons measurement*

Measuring the number of photons is carried out with a Luminotech. The photon sensor has been previously calibrated with standard lactate solutions, plotting the number of photons emitted depending on the number of lactates in the standard solutions.

The analysis is performed on samples of 20µL injected into Ø6mm test tubes. The measurement time is 10 seconds.

C. RESULTS

1) *Release of silicates according to the temperature*

In first time, the analysis was performed using as the marker the silicate. For this, the method has been modified by choosing a luminescent reagent suited to the marker of the considered reaction: silicate (*Figure 2*).

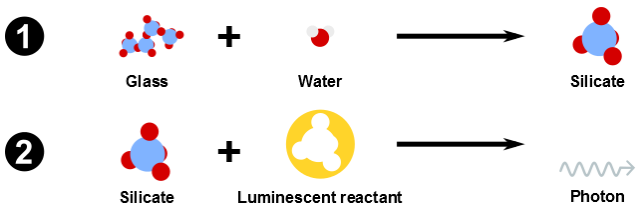

Figure 2: Method Principle (the case of hydrolysis of alterable glasses).

This first series of measures demonstrated the relevance of the method (*Figure 3*).

Without glass, no silicate was detected as well at 37°C than at 65°C (PLA and PLA-NF). Similarly the commercial glass fiber is water resistant (PLA-CF). Furthermore the alterable nature of AF_1 and AF_2 glasses is observed, the first seeming to deteriorate slightly faster than the second. Logically the degradation is greater when the temperature rises.

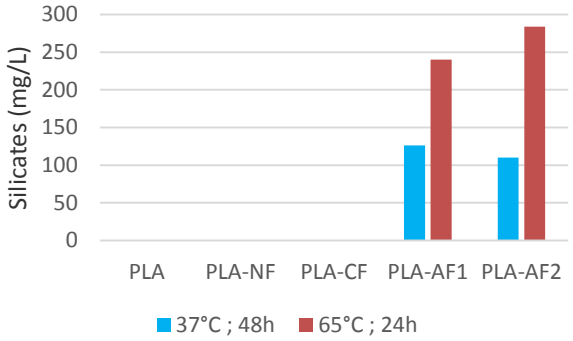

Figure 3: Amount of released silicates (in mg/L) depending on the nature of the reinforcement and of the temperature.

2) *Release of lactates according to the temperature*

Then we measured the degradation in pure water of PLA, the latter being susceptible to hydrolysis (*Figure 4*), mainly for temperatures above its glass transition temperature (≈70°C). This is crucial to distinct the influence of water from that of enzymes latter in this article.

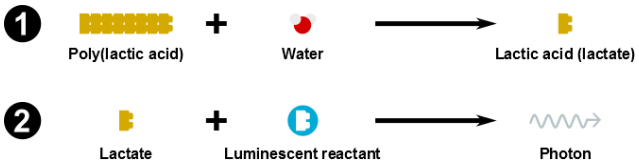

Figure 4: Method Principle (case of the chemical hydrolysis of the PLA).

Whatever the temperature, the chemical hydrolysis of PLA (unfilled or filled with hemp fibers or commercial glass fibers) is null (*Figure 5*).

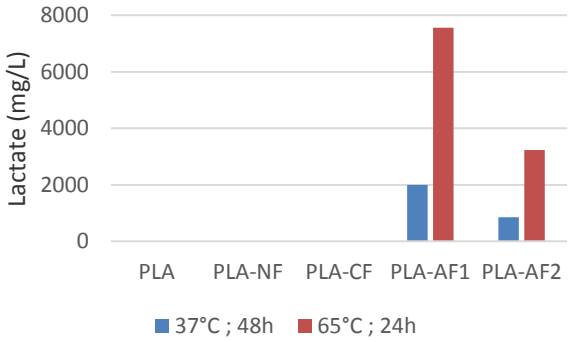

Figure 5: Amount of released lactate (mg/L) depending on the nature of the reinforcement and the temperature.

In the presence of alterable glass fibers the hydrolysis of PLA is active and will vary depending on the glass composition and the temperature. The degradation of PLA is greater in the presence of the fiber AF_1 that the fiber AF_2. Furthermore, the influence of the temperature is similar in both cases, the ratio between the lactate value released at 37°C for 48 hours with respect to that measured after 4h at 65°C being identical and equal to 3.8.

These results show that the nature and composition of the filler may change the water resistance of PLA.

3) *Release of lactates according to the medium*

The presence of a biomimetic enzyme cocktail catalyzes the hydrolysis of PLA and increases the amount of released lactate (*Figure 1*).

As for the abiotic medium tests, differences are observed depending on the nature of the reinforcement (*Figure 6*).

The value of 1800 mg/L obtained when the PLA is reinforced by the commercial glass fiber (PLA-CF) is logical. It is approximately equal to 70% of the value obtained for the PLA alone which corresponds to the mass fraction of

PLA in the composite.

The low value of lactate for the PLA-NF composite is attributed to the decrease in the enzymatic activity of PLA. Measurement using glucose marker type could provide complementary information (glucose is the monomer of the cellulose).

Finally, the release of lactate in the case of composites based on alterable glass fibers is highest.

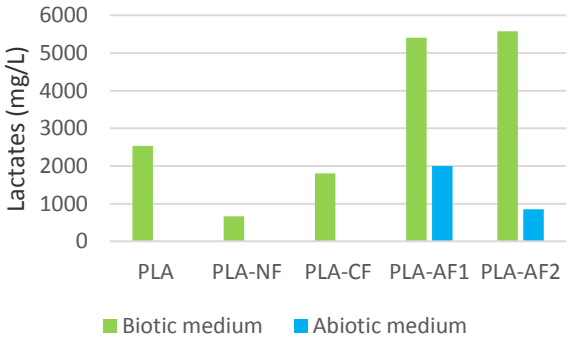

Figure 6: Amount of released silicates (in mg/L) depending on the nature of the reinforcement and the environment.

Furthermore, the difference between the number of lactates in the biological environment and the number of lactates in the abiotic environment relative to the weight fraction of PLA provides additional guidance (*Figure 7*).

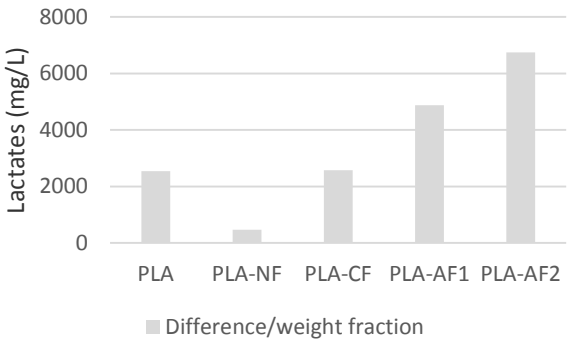

Figure 7: Differences in amounts of silicates released in biotic and abiotic environment reported to the weight fraction of PLA.

The main information provided by this graphic is that alterable glasses accelerate the kinetics of enzymatic degradation of PLA. A buffer silicates (anions) limiting acidification of the medium due to lactate (thus preserving the optimum pH of the enzymes) is for now the preferred scenario in addition to the increase in enzyme/material specific surface area.

D. CONCLUSIONS

The study presented in this article has helped to highlight the relevance of the method for monitoring degradation by chemiluminescence. The enzyme cocktail prepared for this study showed effectiveness. In pure water at 37°C and 65°C (and for a relatively short time), the hydrolytic degradation of PLA is too small to be observed whereas it is high in the presence of enzymes.

Differences were observed depending on the environment (with or without enzymes) and the temperature. Measurements were performed on two completely different materials:

- an organic material (biopolymer): poly (lactic acid);
- an inorganic material like glass alterable by water.

Differences in water sensitivity (conditions of use) as well as biodegradation rate (end of life conditions) were identified based on the type of reinforcement (glass, hemp).

Depending on requirements, this method is adaptable to other materials. It has already been successfully used for:

- the study of other biopolymers degradation: Poly (hydroxybutyrate) (PHB) and starch;
- the measure of cellular activity, by assaying the ATP (adenosine triphosphate);
- etc.

** Founded in 2000 Yelen is a company engaged in the field of quick and easy detection by luminescence (biological / chemical) of substances in trace amounts.*
Application domains range from the food to industry with an adaptation of the method in each case: create / search for a specific reagent and development of a suitable measurement protocol.