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## BIOMASS IMAGING AS A TOOL FOR ADDRESSING THE CHALLENGE OF MULTIPLE-PRODUCT GUAYULE BIOREFINERY

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R & D on guayule (*Parthenium argentatum* Gray) (G) has been driven for a century for securing rubber production (or polyisoprene, PI), a strategic feedstock for tire and glove industry. Sustainable development of G needs an efficient valorization of compounds in bark, wood, leaves, bringing an opportunity to apply the biorefinery concept. However to our knowledge there is no large scale G multiple-product chain. Bulk rubber can be extracted with solvent processes, or a latex can be obtained with an aqueous process enabling to feed tire and glove industry. Solvent processes use ground dry biomass which can be stored prior to extraction, whereas the aqueous process is based on grinding fresh biomass. These processes were set in Mexico and the United States where G is cultivated. G is now being acclimated in Southern Europe; CIRAD and CTTM are optimizing wet grinding steps for high quality latex production (patent application) towards an integrated “green” biorefinery. The chemical composition of EU G biomass (PI 8 %dw, resin 10%dw; total ~2 t/ha) confirms it as a suitable feedstock for producing a range of compounds (PI, terpenes, wax), but their extraction with water (for avoiding harmful solvents) brings a real challenge. Indeed while in hevea, rubber is located in laticifer ducts, G stores it inside cells, but resin is in ducts. To better understand biomass cellular structure and to map in situ biochemical compounds compartmentation and to study the effect of biomass processing on rubber, waxes and resin extraction, we combined several imaging technologies (ie wide field or bright field, epifluorescence, multiphoton and real time microscopy). Our imaging approach allowed (i) to provide an accurate 3D description of secondary metabolites cellular compartmentation, and (ii) to describe the in situ dynamics of polyisoprene particles and understand the aqueous extraction process; for example flocculation or coagulation phenomena due to thermal effect upon grinding on the Brownian motion of particles within the cell. It is necessary to deal with conflicting goals: difficult release of PI particles in water (a non-solvent here); physiological, physicochemical stability of multi-phased systems; chemical constraints (degradation). By understanding the behavior of rubber particles under various thermal, chemical and physicochemical conditions, and dealing with the complex structure of biomass it was possible to monitor the extraction process. In a first step, the process (10kg/batch pilot) extracts up to 80% of high Mw rubber; resin is recovered in a second step with supercritical CO<sub>2</sub>. Taken together, our results show that imaging technologies open new routes for biomass processing to develop an integrated green biorefinery.

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