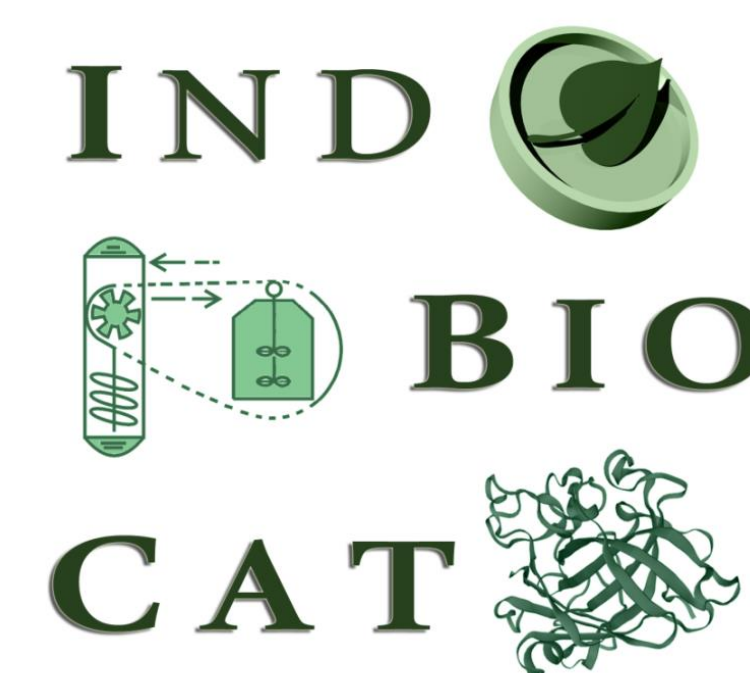




Transglycosylation of steviol glucosides mediated by two novel thermophilic hydrolases, *TtbGal1* and *MtBgl3a*, and valorization of industrial byproducts as sugar donors



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Introduction

Stevia rebaudiana Bertoni is a plant cultivated worldwide due to its use as a sweetener. The sweet taste of stevia is attributed to its numerous steviol glucosides, however, their use is still limited, due to their bitter aftertaste. The transglycosylation of steviol glucosides, aiming at the improvement of their taste, has been reported for many enzymes, however, glycosyl hydrolases are not extensively studied in this respect. **In the present study, a β -glucosidase, *MtBgl3a*, and a β -galactosidase, *TtbGal1*, have been applied to the transglycosylation of two steviol glucosides, stevioside and rebaudioside A.** The maximum conversion yields were 34.6 % and 33.1 % for stevioside, while 25.6 % and 37.6 % were obtained for rebaudioside A conversion by *MtBgl3a* and *TtbGal1*, respectively. Low-cost industrial byproducts were employed as sugar donors, such as cellulose hydrolysate and acid whey for *MtBgl3a*- and *TtbGal1*- mediated bioconversion, respectively. LC-HRMS analysis identified the formation of mono- and di- glycosylated products from stevioside and rebaudioside A. **Overall, the results of the present work indicate that both biocatalysts can be exploited for the design of a cost-effective process for the modification of steviol glucosides.**

Methods

- ✓ Production and purification of recombinant *TtbGal1* and *MtBgl3a*
- ✓ Transglycosylation of steviol glucosides
- ✓ Chromatographic analysis of transglycosylation products
- ✓ LC-HRMS analysis of transglycosylation products

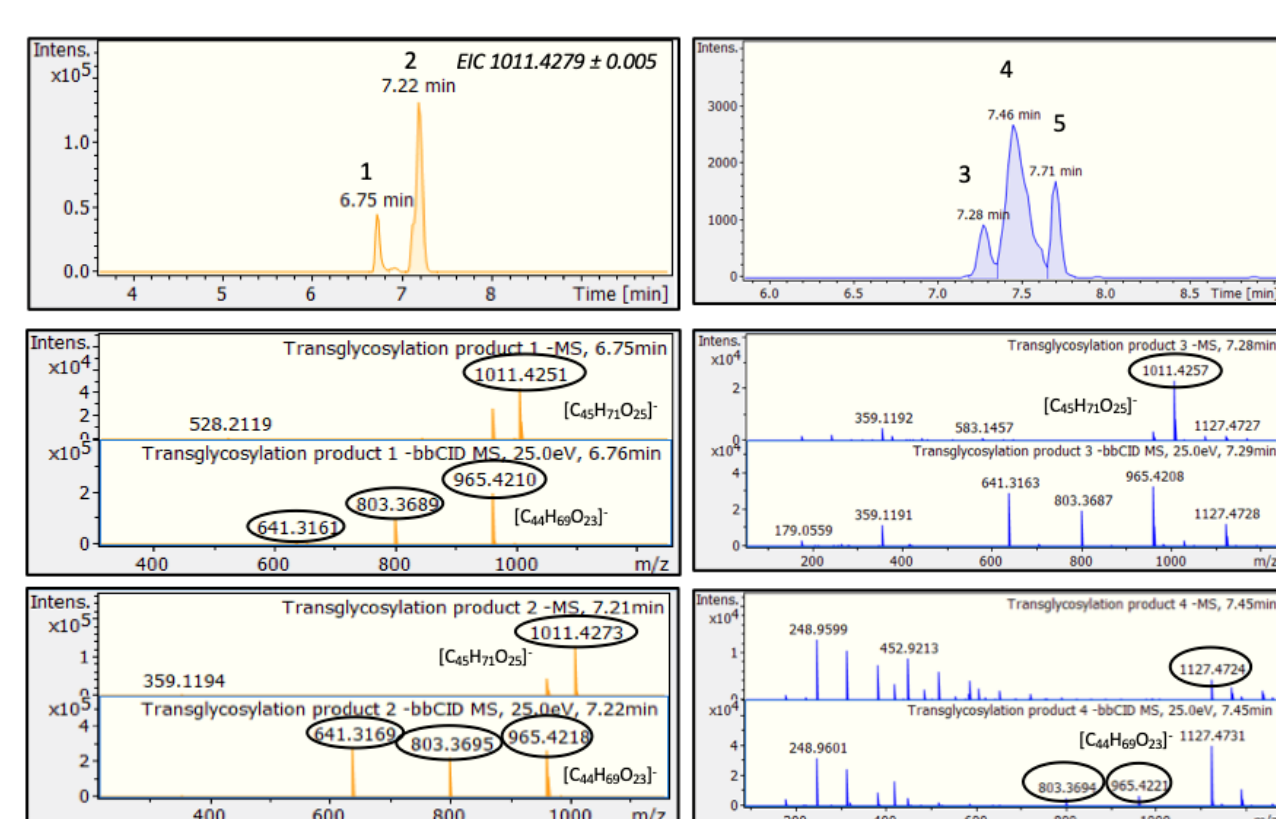
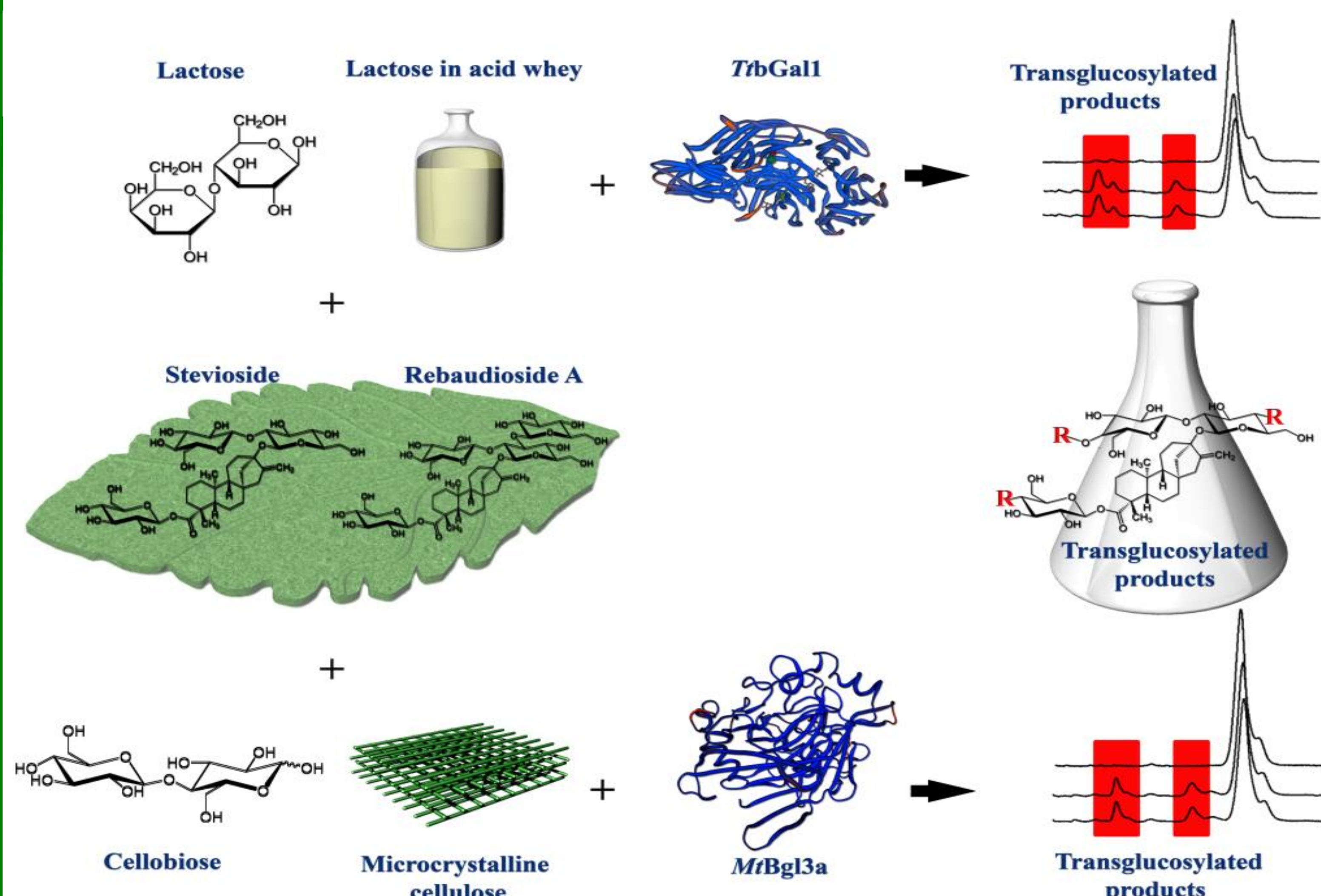
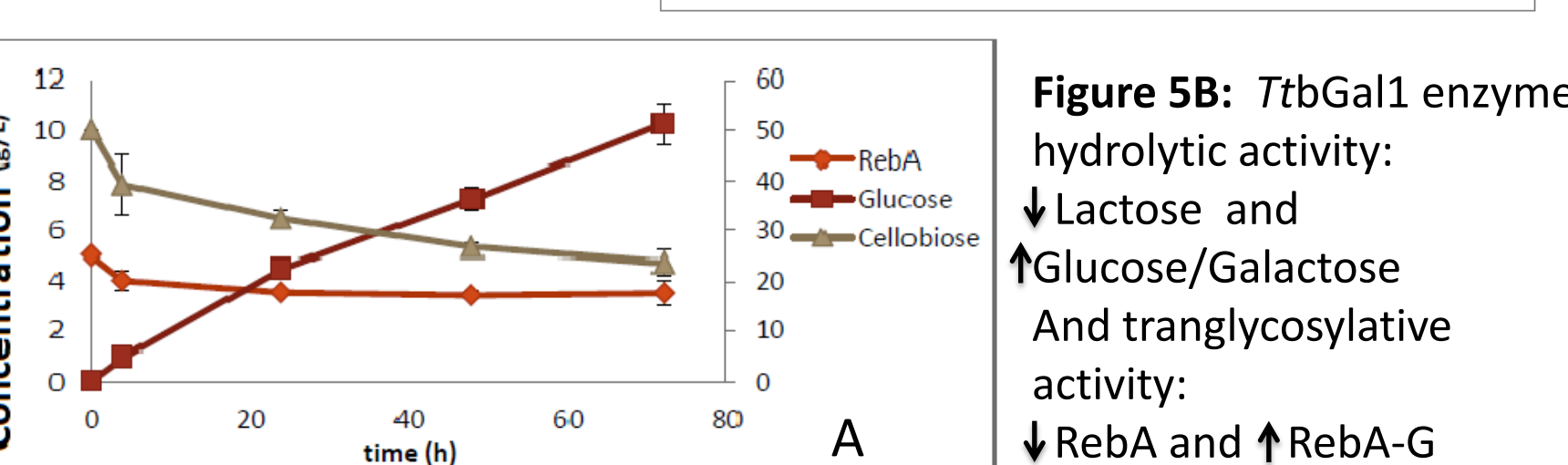
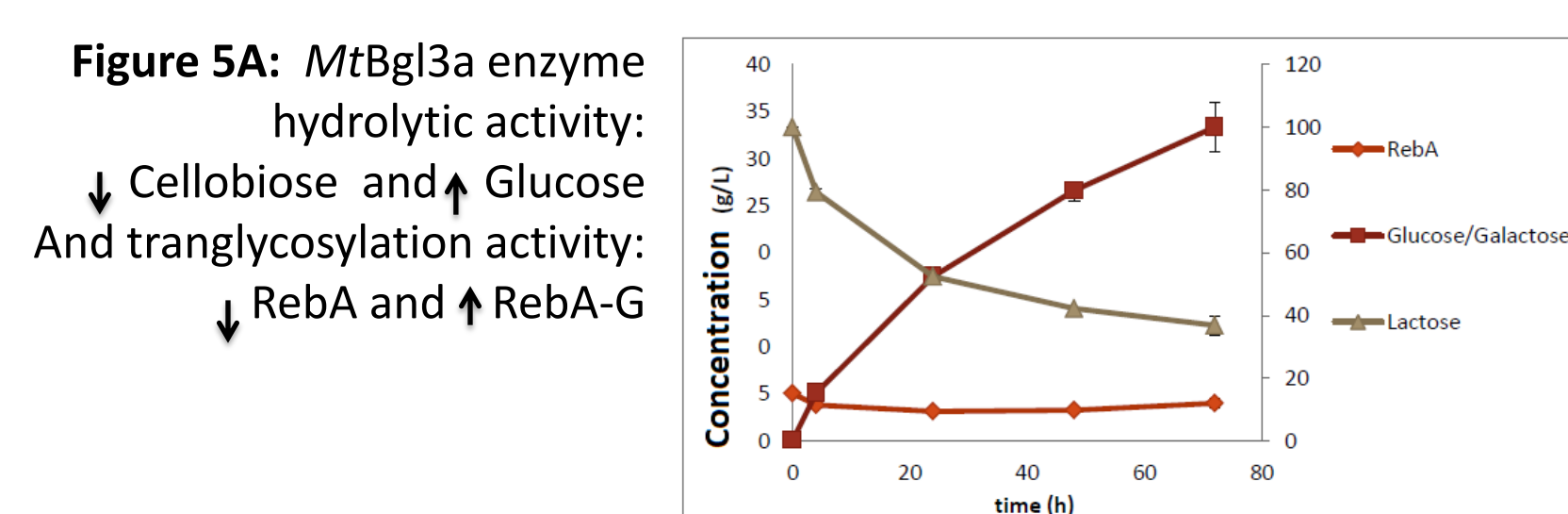


Figure 6: MS and MS/MS spectra of the detected transglycosylated products provided by HILIC-HRMS



Results

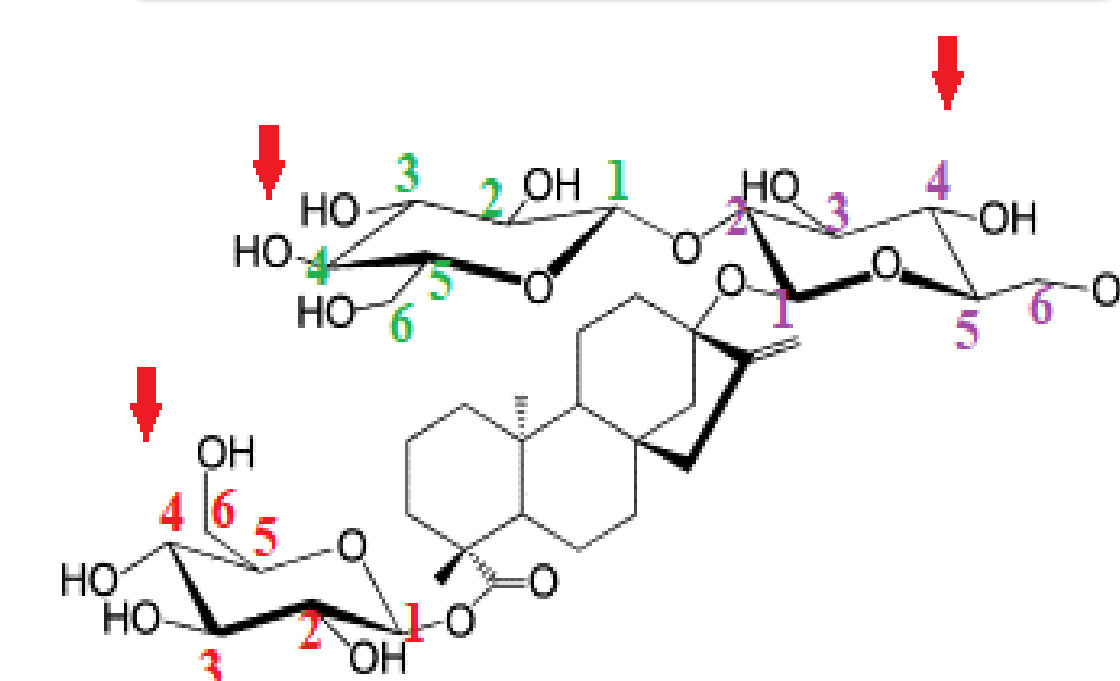


Figure 1: Stevioside and its potential transglycosylation sites.

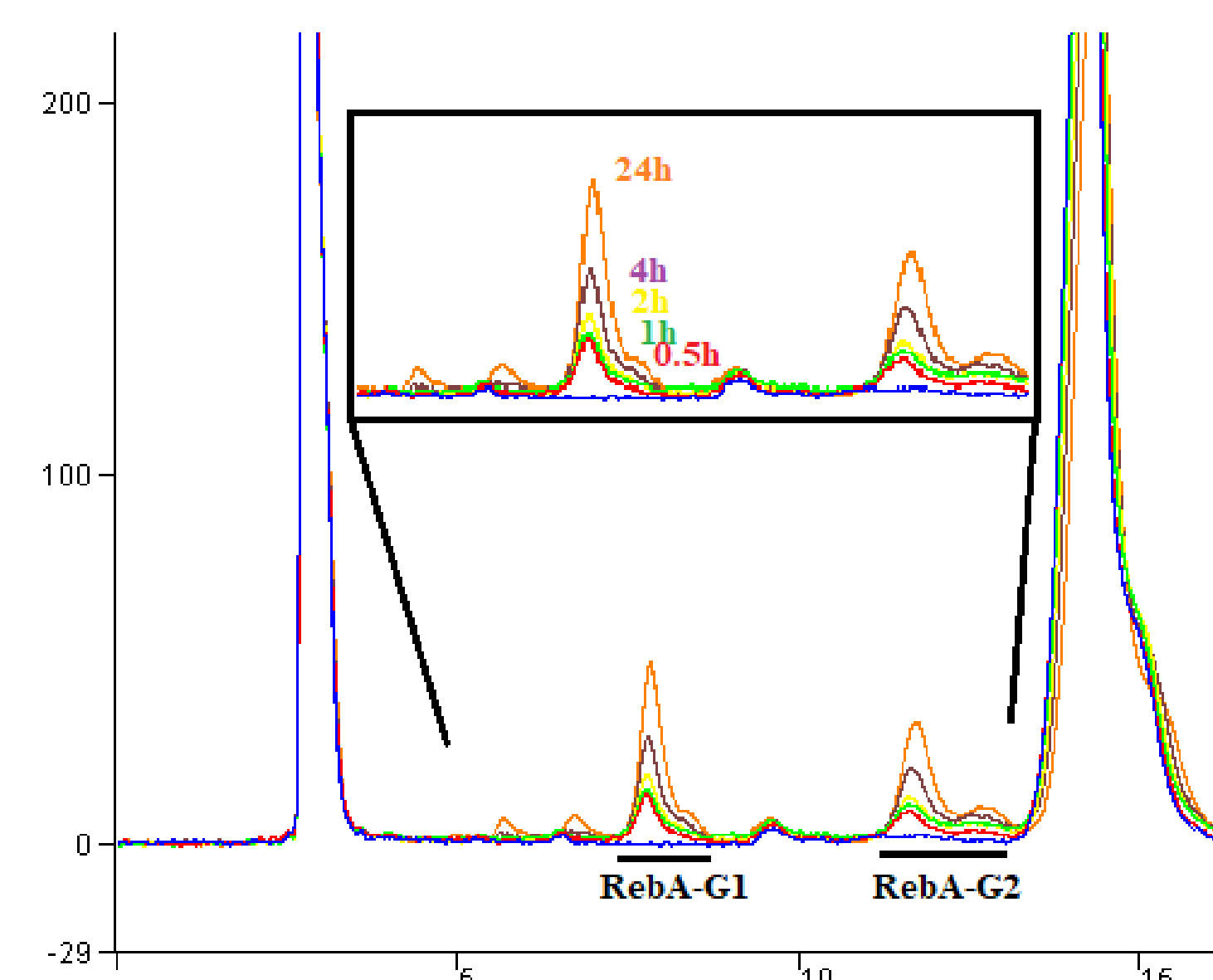


Figure 2: HPLC chromatograph of Rebaudioside A transglycosylated products increasing through time by *MtBgl3a* enzyme.

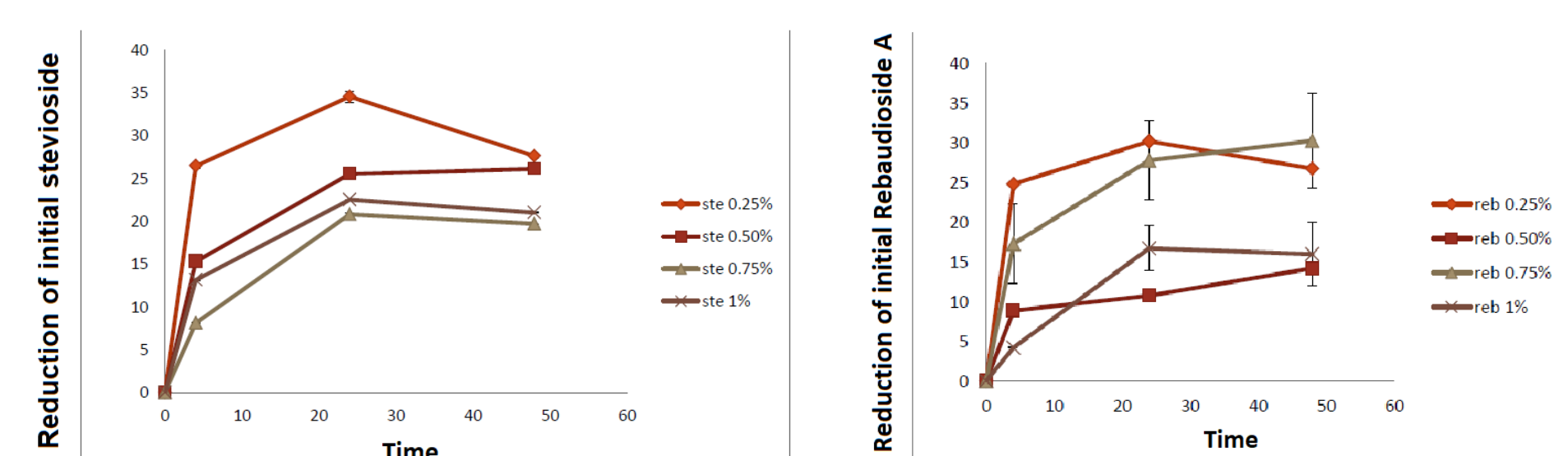


Figure3: The effect of different initial concentrations of steviol glucosides on transglycosylation.

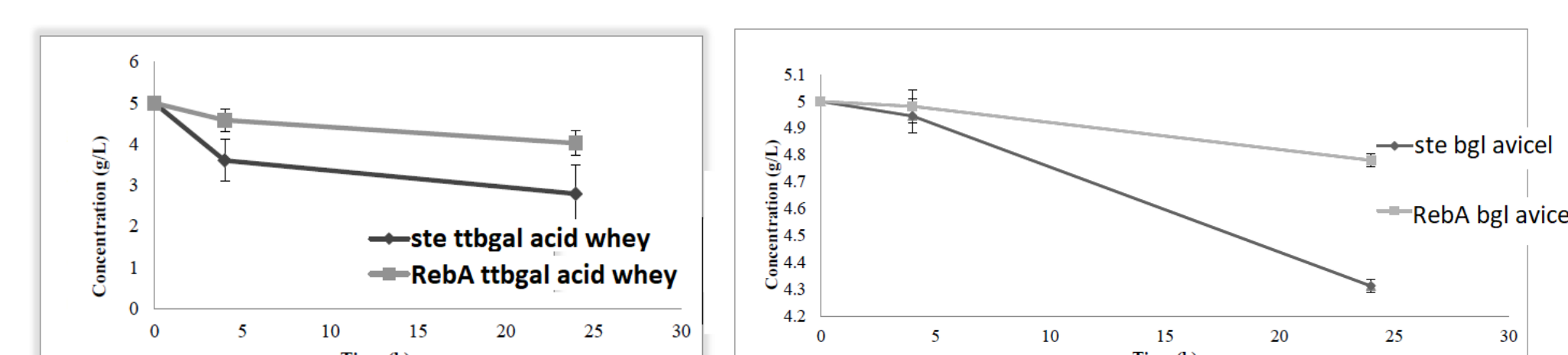


Figure 4: *TtbGal1* and *MtBgl3* hydrolyze acid whey and Avicel, respectively. The released sugars are transglycosylated on steviol glucosides.

Conclusions

- ✓ β -galactosidase *TtbGal1* and β -glucosidase, *MtBgl3a* were applied in the transglycosylation of stevioside and rebaudioside A
- ✓ Both enzymes proved effective in the tested reactions, resulting in very satisfactory conversion yields, which in most cases exceeded 30 %
- ✓ Both bioconversion reactions were performed with low-cost industrial byproducts as sugar donors (Avicel and acid whey), with very promising yields
- ✓ HILIC-HRMS identified the production of mono- and di-glycosylated products

References

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