Enzymatic process for maltotetraose production

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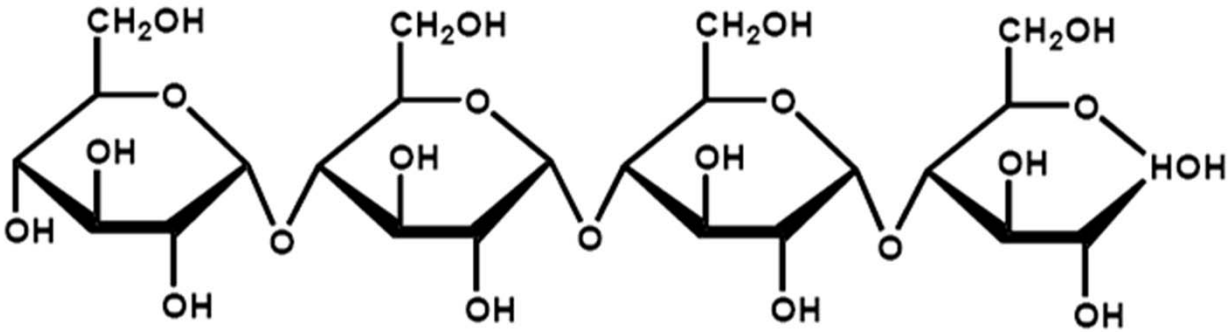
Maltotetraose (G4 or DP4)



Maltotetraose

O-α-D-Glucopyranosyl-[(1→4)-O-
α-D-Glucopyranosyl-]2(1→4)-D-Glucopyranose

DP4-producing alpha-amylase origin

- Origin (from literature)

  Pseudomonas stutzeri

  Pseudomonas saccharophila

  Bacillus circulans

- Specificities (from literature)

  pH— 6.0-7.0

  Temp---50 C

Properties and Applications of DP4 syrup

Properties

- Stable to heat and acid, low Maillard reactivity, colourless
- Mild sweetness (1/3 of sucrose)
- Clean tasting, efficient digestability
- Resistant to retrogradation of starch gel
- Higher moisture retainment at low humidity, lower moisture absorptivity at high humidity than sucrose
- Higher freezing point
- Higher viscosity
- Good tertiary property, inhibits growth of intestinal putrefactive bacteria

Reference

Oligosaccharids: Production, properties and application
Japanese Technology Reviews
Section E: Biotechnology
Vol. 3, No. 2, 1993
Gordon and Breach Science Publishers

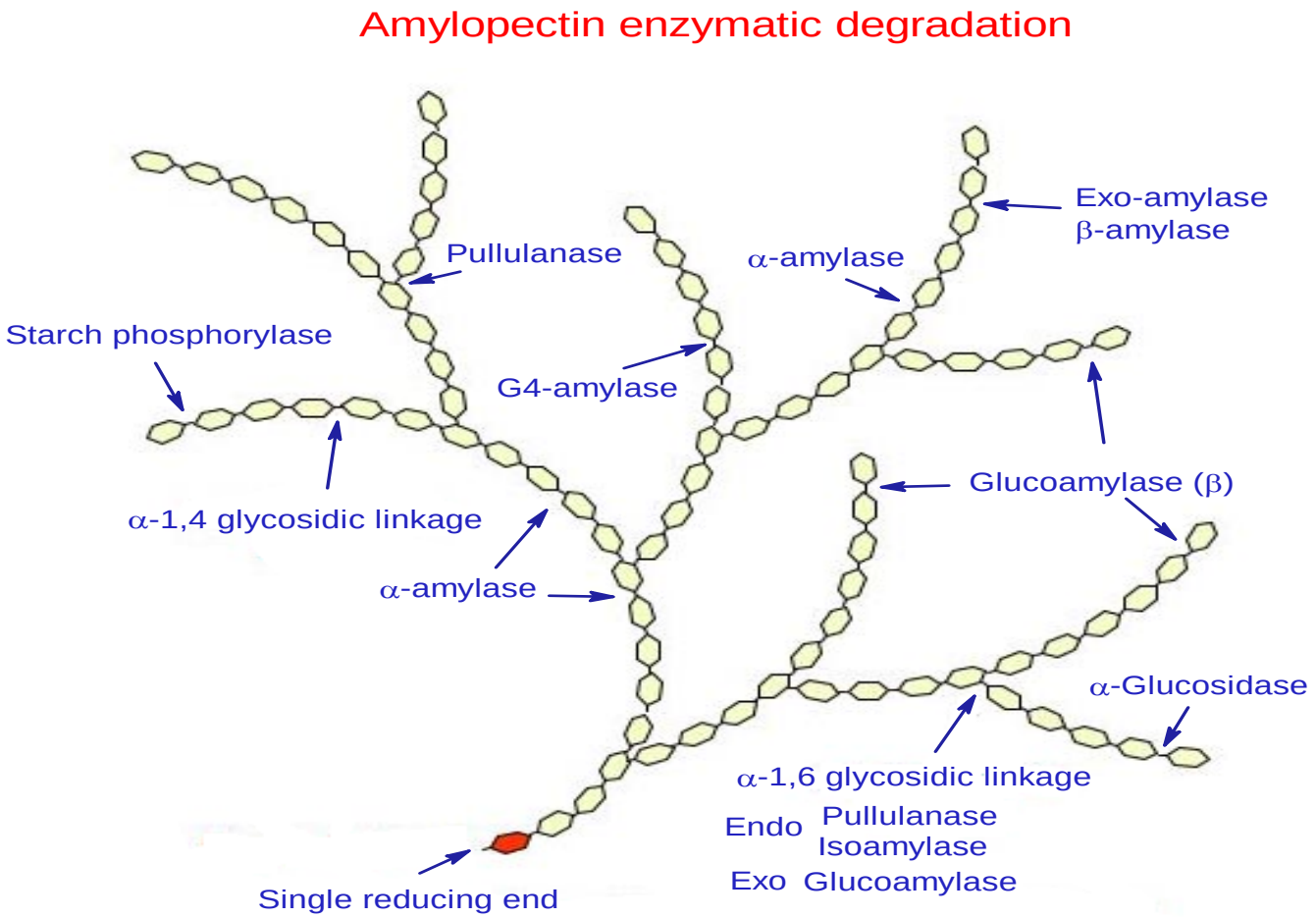
Table 2.
Uses of Maltotetraose Syrup.

Digestibility:	powdered milk, liquid diet.
Low sweetness and good taste in eating:	caramel, ice-cream, jam, butter cream, dressing, candy, ices, jelly, whipped cream, baby foods, Japanese unbaked cakes, Turkish delight, bean-jam cake, soft adzuki-bean jelly, sweet jelly of beans, bean jam, etc., unbaked cakes (custard cream, etc.).
Prevention of hygroscopicity:	hard candy, caramel, chocolate, etc.
Prevention of coloration:	bean jam, cream, fruit jam, hard candy, etc.
Reinforcement agent:	soft drinks, wine, whiskey, seasoning, sauce, soy sauce, tomato ketchup, jam, dressing, flour paste, custard cream, butter cream, jelly.
Glazing and tunicating agent:	rice cracker, dried laver, hard candy, etc.
Regulation of freezing point:	ice-cream, glace ice, frozen foods.
Humectant:	Japanese cakes, unbaked cakes, pastry, soft adzuki-bean jelly, bread, etc.
Powderizing material:	soft drinks, soup, seasoning, coffee whitener, spices.

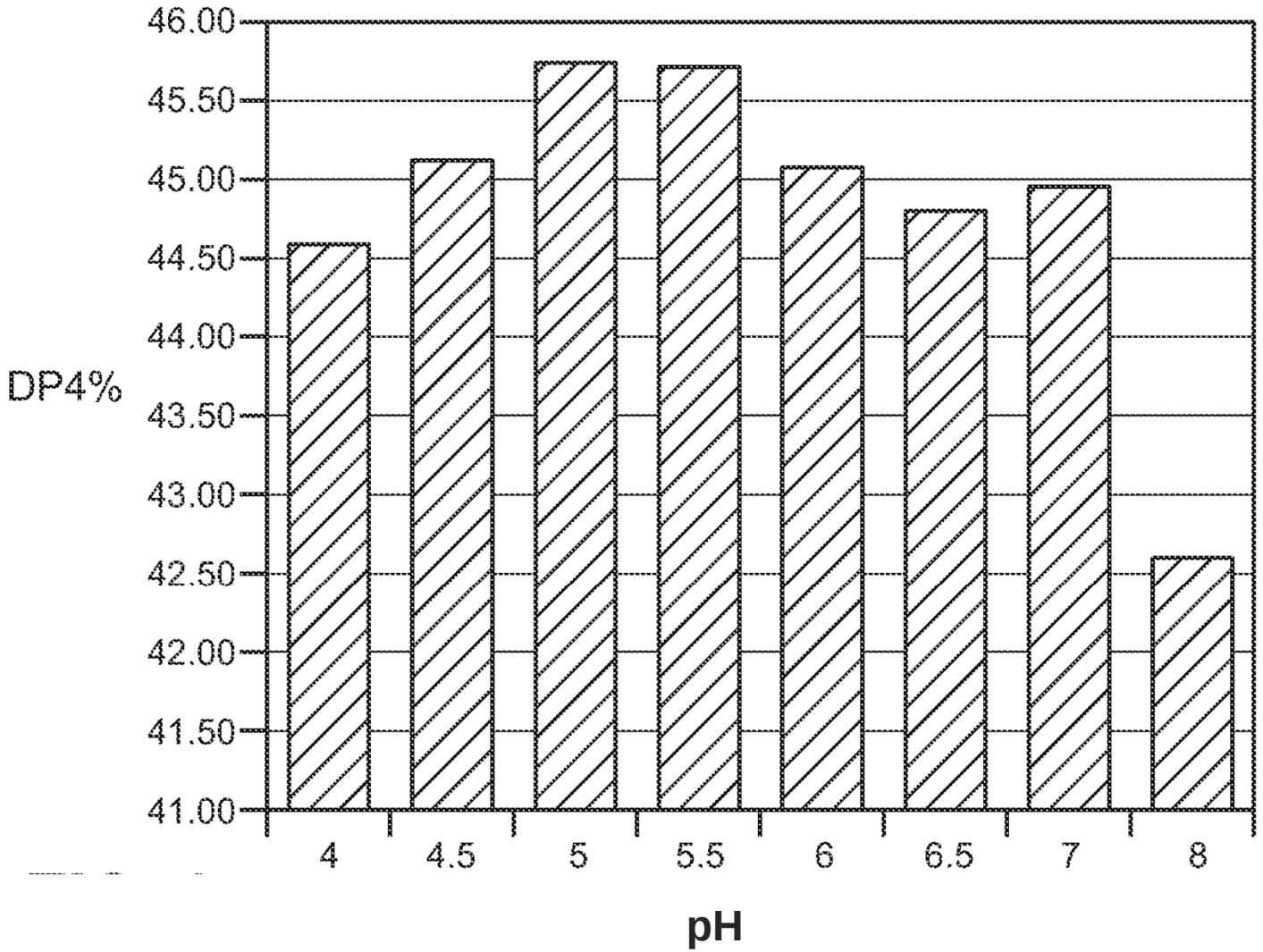
Introduction of SAS3 (DP4-producing alpha-amylase)

- SAS 3 is a genetically modified amylase from *Pseudomonas saccharophilia* expressed in *Bacillus licheniformis*.
- Major product: Maltotetraose
- Exo-type alpha-amylase
- Food-grade (GRAS)
- Ability to hydrolyze conventional liquefied starch to produce high DP4 syrup.

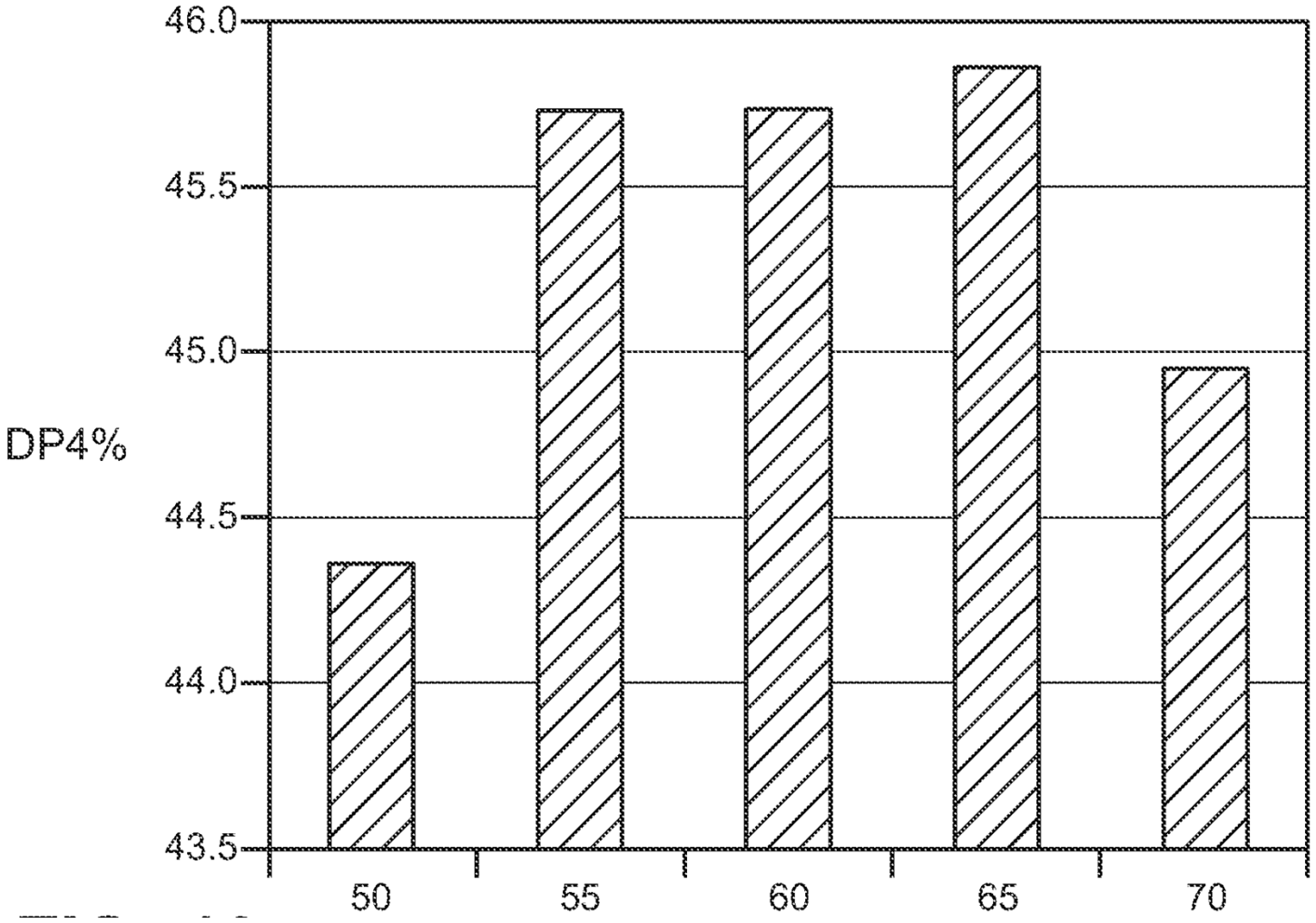
SAS3 : non-maltogenic amylase engineered for thermostability and exo-specificity



pH optima of SAS3



Temperature optima of SAS3

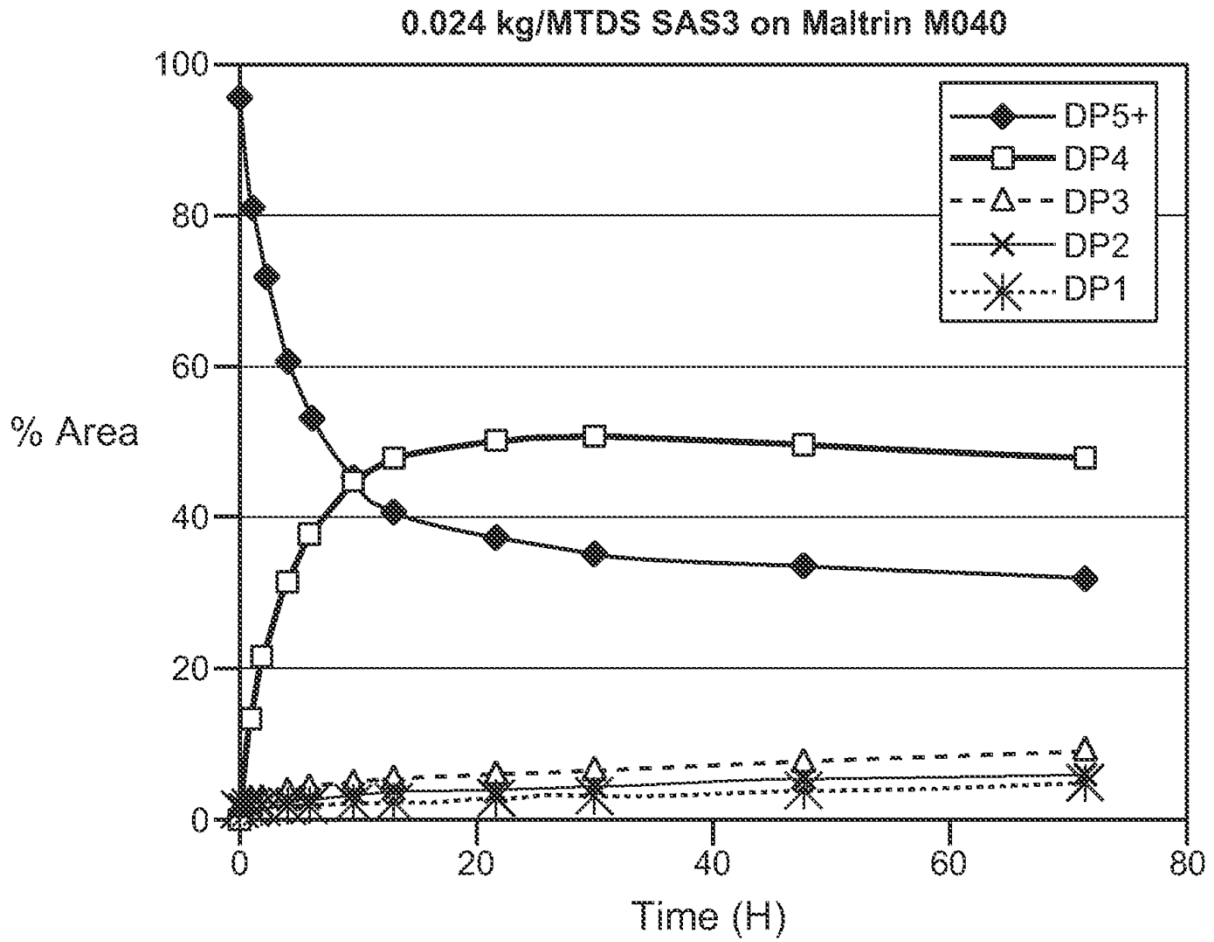


Maltotetraose production

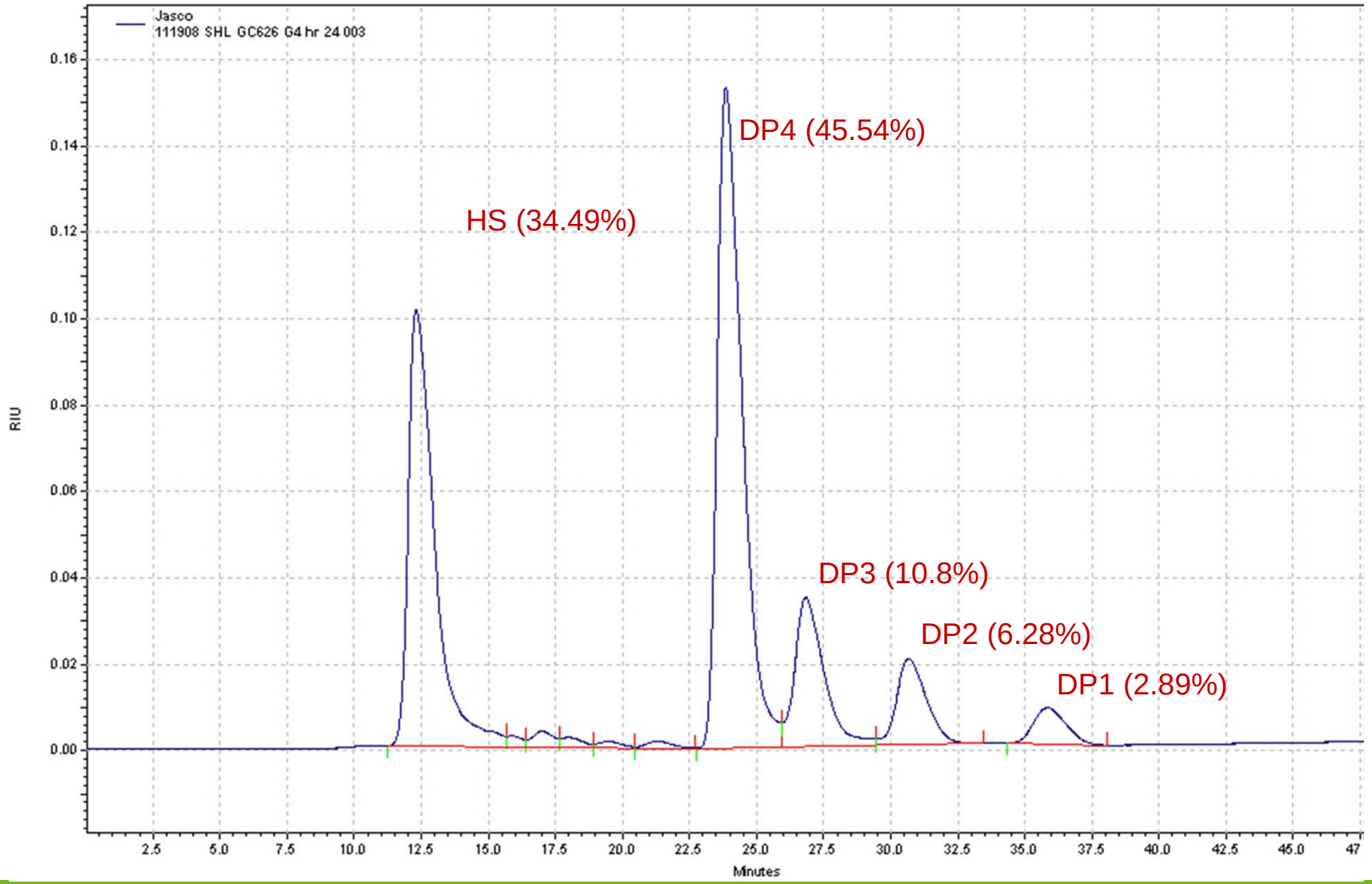
› Maltotetraose production

- Enzyme: SAS 3
- pH: 5.0 ~ 5.5
- Temperature: 60 ^{o}C
- Dosage: 0.024 KG/MTds
- Substrate: Maltodextrin (Maltrin M040) (~32% DS, ~10 DE)
- Target %DP4 > 45%
- Analysis by Rezex ROA-organic acid H+ or RSO-oligosaccharide column (Ag+)

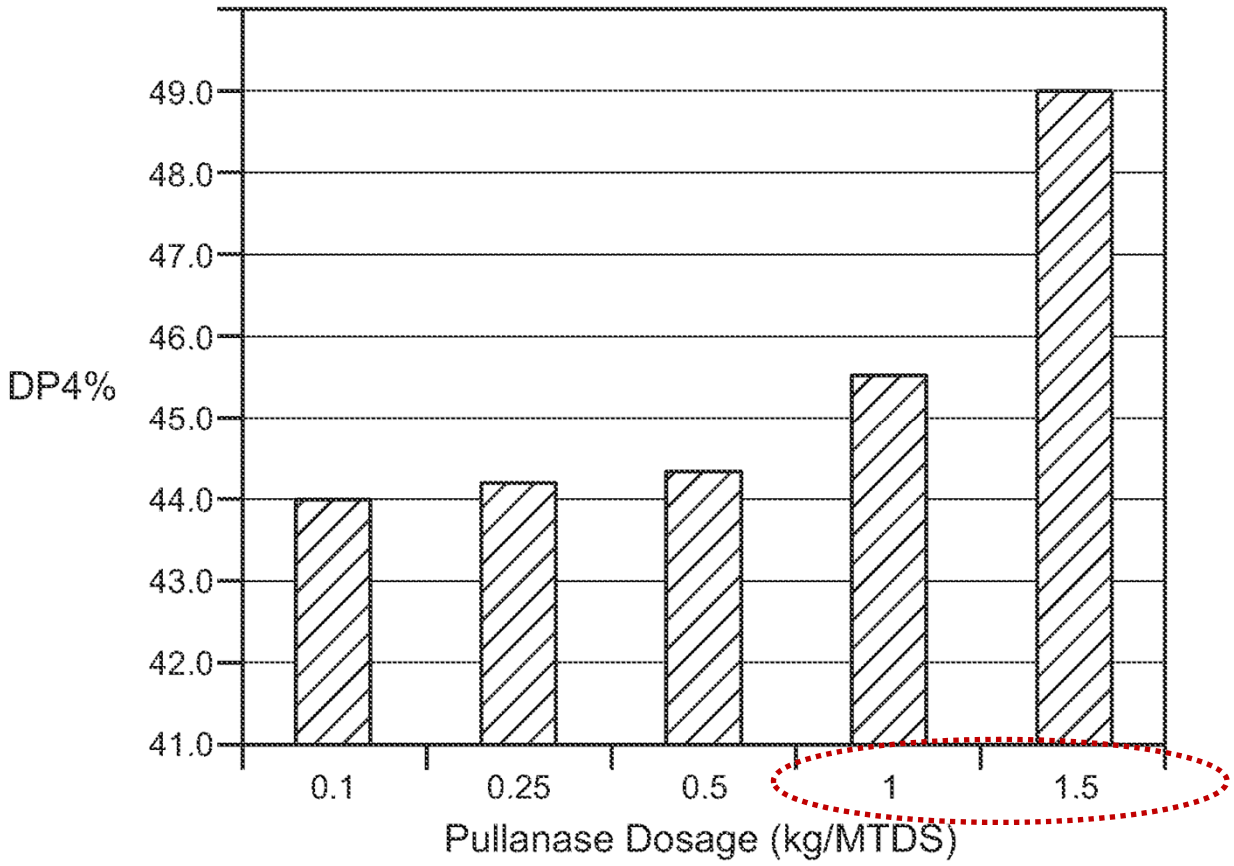
Maltotetraose production



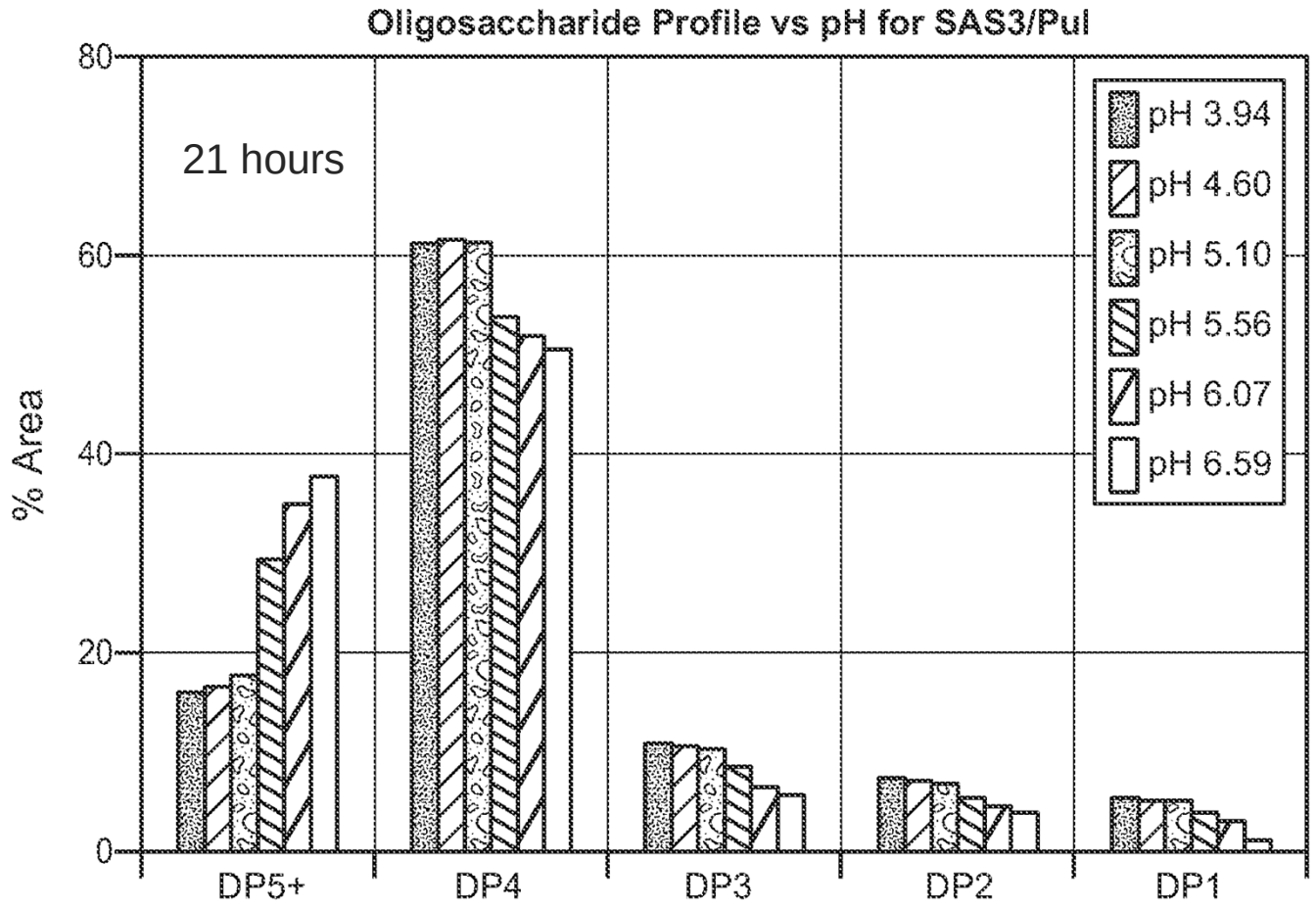
Chromatogram of DP4 syrup



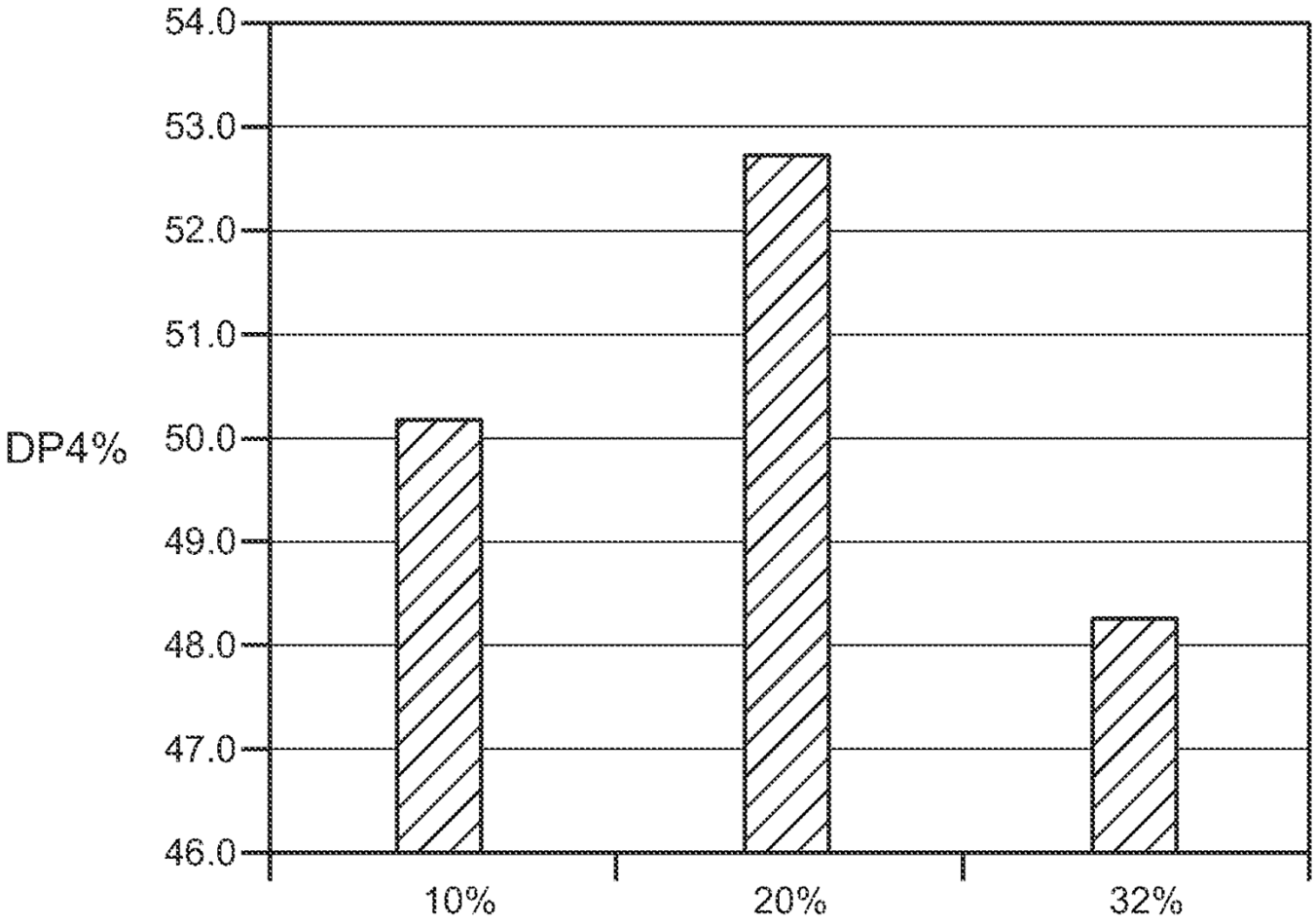
Effect of debrancher
: Optimax® L-1000 (pullulanase)



Effect of debrancher
: pH for SAS3+Optimax® L-1000



Effect of DS



Patent application submitted

- Genencor

"*Production of Maltotetraose Syrup Using a Pseudomonas Saccharophila Maltotetraohydrolase Variant*"

Publication No.: WO2010/118269

Conclusions

- Genetically engineered SAS3 from *Pseudomonas sacharophilia* was expressed in *Bacillus licheniformis*.
- Optimal conditions of SAS3 were pH 5.0~5.5 and 60~65 ^{o}C.
- Incubation of SAS3 with starch resulted in >45% DP4 production.
- Addition of pullulanase was effective to boost DP4 production.
- Lower dry solids (<32%DS) of starch liquefact resulted in increased DP4 production.