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Effect of the Substrate Concentration and Water Activity on the Yield and Rate of the Transfer Reaction of β-Galactosidase from *Bacillus circulans*

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ABSTRACT: Prebiotic galactosyl oligosaccharides (GOS) are produced from lactose by the enzyme β-galactosidase. It is widely reported that the highest GOS levels are achieved when the initial lactose concentration is as high as possible; however, little evidence has been presented to explain this phenomenon. Using a system composed of the commercial β-galactosidase derived from *Bacillus circulans* known as Biolacta FN5, lactose and sucrose, the relative contribution of water activity, and substrate availability were assessed. Oligosaccharide levels did not appear to be affected by changes in water activity between 1.0 and 0.77 at a constant lactose concentration. The maximum oligosaccharide concentration increased at higher initial concentrations of lactose and sucrose, while initial reaction rates for transfer increased but remained constant for hydrolysis. This suggests that the high oligosaccharide levels achieved at the raised initial saccharide concentration are due to increases in reactions that form oligosaccharides rather than decreases in concurrent reactions, which degrade oligosaccharides. There were different effects from changing the initial concentration of lactose compared to sucrose, suggesting that the ability of lactose to act as a donor saccharide may be more important for increasing maximum oligosaccharide concentrations than the combined ability of both saccharides to act as galactosyl acceptors.

KEYWORDS: Galactooligosaccharides, GOS, prebiotic, galactosyl transfer, β-galactosidase, lactose hydrolysis, *Bacillus circulans*, lactosucrose

INTRODUCTION

Oligosaccharides are commercially important food ingredients. They act as "prebiotics", selectively increasing the activity of beneficial, or "probiotic" intestinal microflora[1] to impart health benefits.[2] The estimated global retail market for pre- and probiotic foods grew from U.S. $13.7 billion in 2007 to U.S. $15.4 billion in 2008.[3] Thus, research on the factors that improve the efficiency of oligosaccharide manufacture is of academic and commercial interest.

Galactosyl oligosaccharides (GOS) are an important class of food-grade oligosaccharides produced from dairy lactose by the enzyme β-galactosidase. The reaction for GOS production is under kinetic control[4] and results in a mixture of monosaccharides and prebiotic GOS.

The concentration of GOS within a batch reaction is a function of competition between concurrently occurring pathways that result in either GOS formation or degradation. These can be visualized in the retaining reaction mechanism[5] (Figure 1). A donor saccharide passes a galactosyl unit to transiently form a covalent galactosyl−enzyme intermediate before the pathways diverge. Oligosaccharides are formed when the galactosyl moiety is accepted by another saccharide (e.g., transfer reaction; route 1, 2, or 3 in Figure 1). Alternatively, di- or oligosaccharides are degraded when the acceptor is water (hydrolysis reaction; route 4 in Figure 1). As the reaction continues, oligosaccharides act as galactosyl donors, leading to their degradation when the galactosyl units that they donate are accepted by water. The GOS concentration therefore progresses through a maximum before declining with time.

It has been shown that the maximum concentration of GOS is higher with increased initial concentrations of lactose.[6,7] This is observed for both enzymes with little propensity to form GOS, such as the *Escherichia coli* enzyme[8] and high GOS yielding enzymes.[9,10] This phenomenon also occurs with other enzymatic reactions that form oligosaccharides, such as those catalyzed by β-fructofuranosidases.[11−13]

An explanation for this phenomenon could be less competitive hydrolysis decreasing the degradation of oligosaccharides or more competitive transfer increasing the formation of oligosaccharides. Water activity is reduced at high saccharide concentrations, and it has been hypothesized that this could be responsible for the increased maximum concentration of GOS because it leads to less competitive hydrolysis.[16−18] Alternatively, increases in initial substrate concentration have been hypothesized to make transfer reaction substrates more available, increasing the competitiveness of oligosaccharide formation pathways.[14,15]

The food-grade preparation of β-galactosidase, known as Biolacta FN5, produces a range of GOS structures, although

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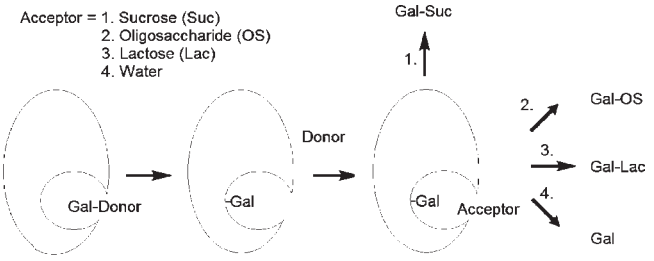

Figure 1. Competing reaction pathways forming and degrading different oligosaccharides from different substrates. Gal, galactose; Suc, sucrose; OS, oligosaccharide; and Lac, lactose.

the major product is 4′-galactosyl lactose [β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-α,β-D-Glc*p*].[19] It was recently found that Biolacta FN5 can catalyze a transfer reaction where lactose is the donor and sucrose is the acceptor to form the prebiotic lactosucrose [β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→2)-β-D-Fru*f*][20] (route 1 in Figure 1). Including sucrose in a reaction allows for a deeper study of the transfer reaction, which requires both a donor and acceptor substrate. Sucrose cannot act as a donor with β-galactosidase; therefore, adding sucrose to the reaction solution can increase the acceptor concentration independently of the donor concentration.

It appears that little work has been performed to compare the relative contributions of water activity and the concentration of substrates for transfer reactions toward increases in oligosaccharide yield in food-grade reaction systems. This paper explores the role of these factors on the maximum oligosaccharide concentration produced from lactose or lactose and sucrose by Biolacta FN5.

MATERIALS AND METHODS

Enzymes and Chemicals. The β-galactosidase preparation derived from *Bacillus circulans*, known as Biolacta FN5, was kindly supplied by Vitachem (Parramatta, Australia). Analytical-grade lactose and D-galactose (Sigma-Aldrich, Sydney, Australia), sucrose and D-glucose (Chem Supply, Gillman, Australia), and organic solvents (Merck, Kilsyth, Australia) were used. Milli-Q water (resistivity < 18.2 Ω) was used for all experiments.

***β*-Galactosidase Reactions.** Reactions were performed in 100 mM sodium acetate buffer at pH 6.6 and a temperature of 40 °C (±0.1 °C). Reactions with a total volume of 10 mL were initiated by the addition of Biolacta FN5 solution containing 0.1 unit of *para*-nitrophenol galactoside activity, as previously described.[22]

The initial reaction rate was determined by sampling within the first 5 min following Biolacta addition, because the initial rates of trisaccharide and galactose formation were found to be linear with respect to time over this period. Each reaction condition was tested in triplicate, sampling at least 3 times during the initial 5 min period.

Maximum oligosaccharide concentrations were obtained by sampling reactions over time until the concentration of GOS either decreased or did not significantly increase over 1 h.

A previously described solvent extraction procedure[23] was used to quench the reaction and prepare samples for analysis by high-performance liquid chromatography (HPLC).

Measurement of Saccharide Concentrations. HPLC was used to analyze saccharide concentrations and was performed with two 300 × 7.8 mm Rezex RCM-Monosaccharide Ca^{2+} columns (Phenomenex) joined in series. The system and other parameters are as described previously.[24] Oligosaccharides were defined as those with a degree of polymerization of 3 or higher, and these were well resolved from disaccharides, as were monosaccharides from disaccharides. However, disaccharides were not resolved from each other with this column that has its highest selectivity for separating monosaccharides. Consequently, sucrose and lactose were not quantified when they were present together in solution. It is likely that transfer products, such as galactobiose and allolactose, co-eluted with lactose and sucrose.

The concentration of saccharides was calculated by interpolation from external standards. Authentic standards were used for lactose, glucose, and galactose. GOS are commercially available only as mixtures; therefore, their concentrations were calculated as raffinose equivalents from an external raffinose standard. In water activity experiments, substantial interference was observed in the region of the chromatogram where GOS eluted; therefore, GOS was quantified by mass balance (GOS = initial lactose − measured lactose − measured glucose − measured galactose).

Data Analysis. The yield of oligosaccharides is expressed in two ways: yield from initial lactose and yield from total saccharides. The yield from initial lactose was the maximum oligosaccharide concentration in percent (w/v) divided by the initial lactose concentration in percent (w/v) multiplied by 100, while the yield from total saccharides was the maximum oligosaccharide concentration in percent (w/v) divided by the total initial saccharide concentration in percent (w/v) multiplied by 100.

The initial reaction rates were calculated as the gradient of linear regression fit to the concentration of saccharides plotted against the reaction time using Microsoft Excel.

Michaelis−Menten constants (V_{max} and K_m) and standard deviations of these constants were determined using MatLab (The MathWorks, Inc., Natick, MA) to regression fit the Michaelis−Menten equation for one substrate to plots of the initial rate against the initial saccharide concentration.

Statistical significance (e.g., $p > 0.05$) was determined by the two-tail Student *t* test assuming equal variance, except where use of the one-tail test is indicated.

RESULTS AND DISCUSSION

Effect of the Lactose Concentration on GOS Yield. Commercial, food-grade β-galactosidase preparations generally respond to raised lactose concentrations by producing more GOS,[6,7] and Biolacta FN5 behaves in this way.[25] This expected phenomenon was reproduced with the maximum oligosaccharide concentration increasing across the initial lactose range of 1−20% (w/v) (Table 1). The values reported here are higher than those reported by Boon et al.[25] for the same enzymatic preparation and reaction temperature. It seems likely that this difference was primarily due to Boon et al. quantifying only trimeric oligosaccharides, while here all GOS species were measured, including species with a higher degree of polymerization.

Effect of the Water Activity on GOS Yield. Water activity (a_w) can be used as a measure of the availability of water to participate in an enzyme catalyzed reaction,[26] where $a_w = 1.0$ for pure water and $a_w = 0.0$ for an anhydrous system. High concentrations of lactose in aqueous solution reduce a_w, and it has been hypothesized that this decrease reduces the competitiveness of hydrolysis reactions, leading to higher maximum concentrations of GOS.[16−18]

To test this hypothesis, GOS forming reactions were performed in media with a_w varied by the addition of 0−5 M NaCl or 0−40% (v/v) ethanol at an intermediate lactose concentration of 10% (w/v). Lactose in water has an $a_w = 0.995$ at 10% (w/v), and at the elevated concentration of 25% (w/v), $a_w = 0.982$.[27] The decrease in a_w because of NaCl or ethanol was much more substantial than the decrease due to 10% (w/v) lactose, with an a_w of 0.80 reported for 5 M NaCl in water and an a_w of 0.77 reported for 40% (v/v) ethanol in water.[28]

Table 1. Increasing GOS Yields with an Increasing Initial Lactose Concentration

this study			ref 25			
initial lactose concentration (%, w/v)	maximum GOS concentration[a] (%, w/v)	GOS yield (%, w/w)	initial lactose concentration (mol/kg)	initial lactose concentration (%, w/v)	maximum GOS concentration (%, w/v)	GOS yield (%, w/w)
1	0.2 (0.02)	18	0.19	6.6	0.8	12
5	1.5 (0.05)	29	0.35	12.5	2.4	19
10	4.2 (0.42)	42	0.49	17.9	3.8	21
20	8.4 (0.48)	42	0.59	21.9	6.9	32

[a] Mean of triplicate reactions with standard deviation given in parentheses.

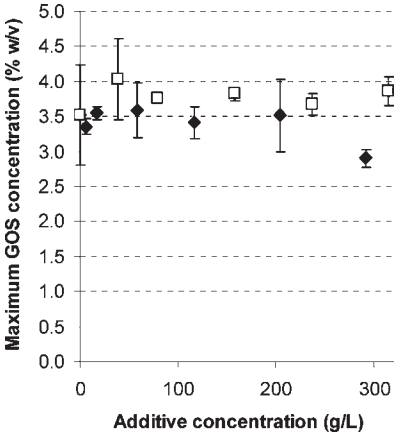

Figure 2. Maximum GOS concentration formed by Biolacta FN5 in a 10% (w/v) lactose solution in the presence of NaCl or ethanol to reduce water activity. Mean of triplicate reactions; error bars depict one standard deviation. GOS concentration calculated by mass balance (see Measurement of Saccharide Concentrations). (◆) NaCl added and (□) ethanol added.

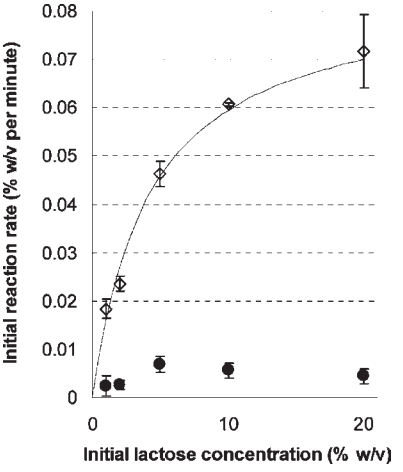

Figure 3. Effect of lactose concentration on the rate of transfer and hydrolysis reactions catalyzed by Biolacta FN5. Mean of triplicate reactions; error bars depict one standard deviation. (◇) Initial rate of trisaccharide formation; line shows regression fit of the Michaelis−Menten equation for one substrate. (●) Initial rate of galactose formation.

The maximum GOS concentration produced was not significantly increased by including concentrations of 0−5 M (0−292 g/L) NaCl or 0−40% (v/v) (0−316 g/L) ethanol in the reaction solution (Figure 2). This indicates that the minor reduction in a_w caused by lactose was unlikely to contribute to the increase in the maximum GOS concentration observed at lactose concentrations up to 25% (w/v) in this study and in the literature.[6,7]

It should be noted that there is evidence that the maximum GOS concentration increases when a_w is decreased more substantially than described here. For example, the yield of GOS from initial lactose was increased from 38% (w/w) in aqueous solution to 45% (w/w) in a cyclohexane/water (95:5, v/v) medium using Biolacta N5 at 55% (w/w) lactose.[29] The a_w = 0.3 in cyclohexane/water (90:10, v/v);[30] therefore, the a_w of the cyclohexane/water (95:5, v/v) medium was substantially lower than the range of a_w studied here.

It is concluded that any reduction in water activity because of lactose did not contribute to increases in oligosaccharide production at high substrate concentrations.

Correlation of Transfer and Hydrolysis Reaction Rates with Maximum GOS Concentration. The complexity in the kinetics of Biolacta-FN5-catalyzed oligosaccharide formation and degradation can be reduced by studying the initial phase of the reaction when lactose is the dominant saccharide present. Under this condition, the net rate of transfer can be estimated as the rate of trisaccharide formation, because no oligosaccharides with a higher degree of polymerization are present. The rate of trisaccharide degradation during this period is assumed to be low because of the low concentration (less than 0.4%, w/v) of these species. The net initial rate of hydrolysis was estimated by the rate of galactose formation, because galactose is liberated when water is the acceptor, regardless of which saccharide serves as the donor.

The initial rate of hydrolysis was reasonably constant between 1% (w/v) and 20% (w/v) lactose (Figure 3), and the hydrolysis rate was not significantly changed because of increases in lactose concentration. This is consistent with the known Michaelis−Menten behavior of lactose hydrolysis by Biolacta FN5,[31] which has a K_m of 41.7 mM or 1.4% (w/v). This concentration is below most of the substrate concentrations tested here, indicating that changes in reaction velocity would be expected to occur on a different scale of lactose concentration.

In contrast, the initial rate of trisaccharide formation increased across the entire range of the lactose concentrations tested (1−20% (w/v); Figure 3). This increase is well-described by the Michaelis−Menten equation for one substrate (R^2 = 0.986). The apparent Michaelis constant (K_m^{app}) was 4.3% (w/v) (±standard deviation of 0.2) (or 126 ± 6 mM), and the apparent maximum velocity (V_{max}^{app}) was 0.085% (w/v) min^{-1} (±standard deviation of 0.002) (or 2.5 ± 0.06 mM min^{-1}).

These results suggest that the increase in the maximum concentration of GOS observed at elevated lactose concentrations (Table 1) is probably influenced more by an increase in the competitiveness of transfer rather than a decrease in the

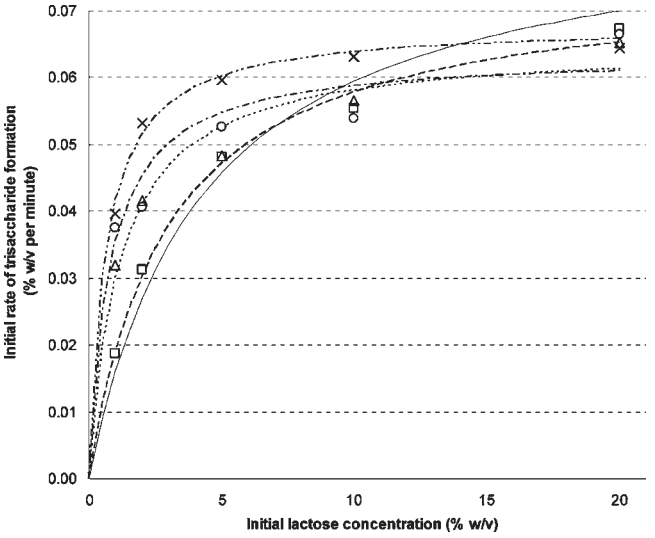

Figure 4. Regression fits of the one substrate Michaelis−Menten equation to the rate of the transfer reaction catalyzed by Biolacta FN5 at varied sucrose concentrations. (—) Michaelis−Menten curve for no added sucrose. (□, - - -) 2% (w/v) sucrose added. (△, · · ·) 5% (w/v) sucrose added. (○, - · -) 10% (w/v) sucrose added. (×, - - · ·) 20% (w/v) sucrose added.

competitiveness of hydrolysis for the commercially relevant range of lactose concentrations tested here. This is consistent with the conclusion from the water activity experiments that reduced hydrolysis was unlikely to be important in the increases in maximum GOS concentration at elevated lactose concentrations.

Effect of Donor and Acceptor Concentrations on Initial Rates of Transfer. The availability of transfer reaction substrates was further examined. The β galactosidase catalyzed reaction involves two substrates: a donor and an acceptor. Lactose fills both of these roles during GOS formation. Sucrose cannot act as a donor with β-galactosidase; therefore, adding sucrose to the reaction solution can increase the acceptor concentration over the donor concentration. The relative contribution of the role played by these two substrates in affecting the transfer rate of Biolacta FN5 was probed by varying sucrose and lactose concentrations.

The concentration of sucrose acting as an acceptor appeared to be important at low donor galactosyl (lactose) concentrations. The transfer rate at low lactose concentrations was significantly increased by adding more acceptors in the form of sucrose (Figure 4 and Table 2). The transfer rate with both 1% (w/v) and 2% (w/v) lactose was significantly increased with added sucrose for all concentrations tested. This suggested that reduced availability of acceptors limited the transfer rate at 1% (w/v) and 2% (w/v) lactose with no sucrose.

The transfer rate became less sensitive to the addition of sucrose as the lactose concentration was increased. At 5% (w/v) lactose, the transfer rate was only significantly increased when high concentrations of sucrose were added (Table 2; 10% (w/v) and 20% (w/v) sucrose). At 10% (w/v) lactose, the increase in the transfer rate was significant only with sucrose added to 20% (w/v). At these higher lactose concentrations, the transfer rate was increased only when the acceptor concentration was increased by a factor of 2 or more.

At 20% (w/v) lactose, none of the increases in the acceptor concentration tested caused a significant increase in the transfer rate (Table 2). This suggests that the enzyme was saturated with acceptors at 20% (w/v) lactose.

The one substrate form of the Michaelis−Menten equation gave a fair fit to transfer rates plotted against the lactose concentration at each added sucrose concentration (Figure 4), despite the simplification implied by this model. The lowest R^2 value was 0.93, observed for the data set with 5% (w/v) added sucrose. The transfer rate was higher at low lactose concentrations when more acceptor was available because of added sucrose. This was reflected in the estimated K_m^{app}, which gives the concentration of lactose at which the transfer rate reaches half of its maximal velocity. This constant decreased with an increased sucrose concentration (Table 2), quantifying the increase in the efficiency of the transfer reaction caused by added sucrose at low lactose concentrations.

Lactose changed the rate of transfer across a wider range of concentrations than sucrose. Changes in the lactose concentration between 1 and 10% (w/v) resulted in significant increases in the transfer rate at all sucrose concentrations tested, including the highest sucrose concentration (20% (w/v); Figure 4). This was in contrast to reactions containing 20% (w/v) lactose, which did not have a significantly different transfer rate at any of the sucrose concentrations tested. This demonstrates that the transfer rate is more sensitive to the lactose concentration than the sucrose concentration, and it seems likely that this reflects a greater importance in the donor availability.

Effect of Donor and Acceptor Concentrations on Transfer Reaction Product Yields. The maximum oligosaccharide concentration and oligosaccharide yield were measured at varied concentrations of lactose and sucrose.

The presence of excess sucrose (20%, w/v) was found to significantly increase the maximum oligosaccharide concentration achieved at all lactose concentrations examined (□ in Figure 5). As was the case for the transfer rate, increases in the maximum oligosaccharide concentration were particularly substantial at the lower lactose concentrations examined. For example, increases of 6.5- and 3.5-fold in oligosaccharide concentrations were observed at 1% (w/v) and 5% (w/v) lactose, respectively, in the presence of 20% (w/v) sucrose compared to when it was absent.

The addition of excess sucrose, however, was an inefficient means of increasing the oligosaccharide yield from total initial saccharides. The presence of 20% (w/v) sucrose gave a lower yield of oligosaccharides from the total initial saccharides compared to reactions with the same lactose concentration and no added sucrose. It was clear that much of the 20% (w/v) sucrose remained unreacted when the maximum oligosaccharide concentration had been reached, although HPLC peaks for sucrose and lactose were not sufficiently resolved to quantify their concentration.

The effect of the ratio of lactose/sucrose on the maximum oligosaccharide concentration and yield was assessed by maintaining the total initial saccharide concentration at 20% (w/v) and altering the ratio of the mass of lactose/sucrose to 1:2, 1:1, or 2:1. The maximum concentration of oligosaccharides increased with the lactose concentration (△ in Figure 5). For example, 8.4 ± 0.5% (w/v) oligosaccharides were produced from 20% (w/v) lactose and no sucrose compared to 5.8 ± 0.1% (w/v) oligosaccharides from 10% (w/v) lactose and 10% (w/v) sucrose. This finding was in contrast to the optimal ratio of 1:1 lactose/sucrose reported for the formation of lactosucrose isomers [β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→2)-β-D-Fru*f* and β-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→2)-β-D-Fru*f*].[20] It appears that the substrate

Table 2. Rates of Transfer Catalysed by Biolacta FN5 with Added Sucrose

sucrose concentration (%, w/v)	lactose concentration (%, w/v)	mean[a] rate of trisaccharide formation [% (w/v) min^{-1}]	K_m^{app} [b] (%, w/v)	V_{max}^{app} [b] [% (w/v) min^{-1}]
0	1	0.011 ± 0.002	4.3 ± 0.1 (126 ± 3 mM)	0.085 ± 0.003 (2.5 ± 0.1 mM min^{-1})
	2	0.023 ± 0.002		
	5	0.046 ± 0.003		
	10	0.061 ± 0.000		
	20	0.075 ± 0.008		
2	1	0.019 ± 0.002	2.9 ± 0.5 (85 ± 15 mM)	0.075 ± 0.004 (2.2 ± 0.1 mM min^{-1})
	2	0.031 ± 0.002		
	5	0.048 ± 0.005		
	10	0.055 ± 0.008		
	20	0.067 ± 0.003		
5	1	0.032 ± 0.007	1.2 ± 0.3 (35 ± 9 mM)	0.065 ± 0.003 (1.9 ± 0.1 mM min^{-1})
	2	0.042 ± 0.011		
	5	0.048 ± 0.007		
	10	0.057 ± 0.003		
	20	0.065 ± 0.004		
10	1	0.038 ± 0.001	0.8 ± 0.2 (23 ± 6 mM)	0.063 ± 0.003 (1.8 ± 0.1 mM min^{-1})
	2	0.041 ± 0.003		
	5	0.053 ± 0.002		
	10	0.054 ± 0.002		
	20	0.066 ± 0.008		
20	1	0.040 ± 0.006	0.6 ± 0.1 (18 ± 3 mM)	0.068 ± 0.002 (2.0 ± 0.1 mM min^{-1})
	2	0.053 ± 0.002		
	5	0.060 ± 0.002		
	10	0.063 ± 0.001		
	20	0.064 ± 0.008		

[a] Mean of triplicate reactions shown ± standard deviation. [b] Constants of one substrate form of the Michaelis−Menten equation estimated by regression analysis ± standard deviation. The same constants are given in mM for K_m^{app} and mM min^{-1} for V_{max}^{app} in parentheses.

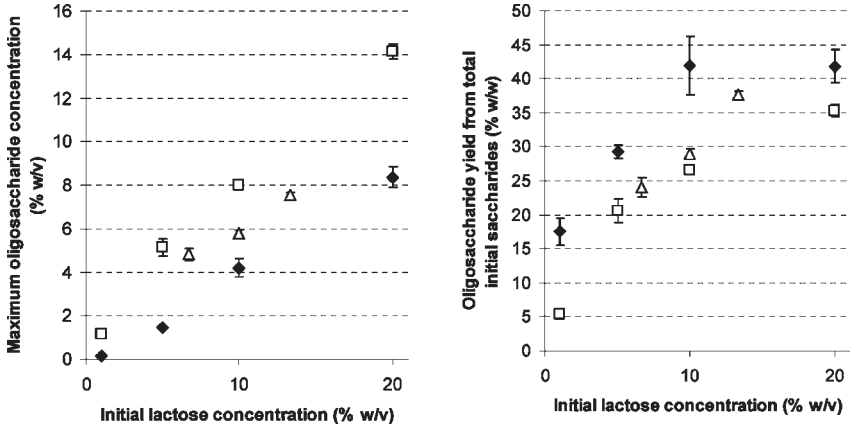

Figure 5. Maximum oligosaccharide concentration and yield observed at varied initial lactose concentrations. Mean of triplicate reactions; error bars depict one standard deviation. (◆) Lactose only. (□) Lactose plus 20% (w/v) sucrose. (△) 20% (w/v) total saccharide, e.g., 6.6% (w/v) lactose and 13.4% (w/v) sucrose (1:2 mass ratio), 10% (w/v) lactose and 10% (w/v) sucrose (1:1 mass ratio), and 13.4% (w/v) lactose and 6.6% (w/v) sucrose (2:1 mass ratio).

concentration ratio, which is optimal for producing specific lactosucrose species, is different to the ratio that generates the most total oligosaccharide.

These experiments demonstrate that, in this system, sucrose cannot replace lactose without reducing the oligosaccharide yield possible from the total initial concentration of these saccharides. This suggests that lactose acting as the donor substrate may be more important than the acceptor substrates, lactose and sucrose, for enabling transfer reactions to compete effectively with concurrent hydrolysis reactions for Biolacta FN5. This is in agreement with the conclusions drawn from the transfer rate data.

These results suggest that sucrose could be added to enhance the maximum concentration of oligosaccharides that can be

achieved with a set and relatively low concentration of lactose. The presence of additional sucrose in this situation would also increase the achievable yield of oligosaccharides from this initial lactose concentration. Such a scenario could occur when dairy derived products are used as a source of lactose, because these typically contain approximately 5% (w/v) lactose. Here, 20% (w/v) sucrose increased the yield of oligosaccharides from 5% (w/v) lactose (Figure 5). This concentration of sucrose would be unusually high in most dairy products but it seems likely that lower concentrations that are commonly used as sweeteners (ca. 5%, w/v) would increase the levels of oligosaccharides produced.

It remains to be tested if the product mixture obtained by adding sucrose to a Biolacta FN5 reaction offers higher prebiotic efficacy than GOS produced from lactose alone. The mixture produced when sucrose is added contains two isomeric lactosucrose products.[20] Lactosucrose oligosaccharides are commercially important prebiotics, although historically produced at lower volumes than GOS (1600 tons, cf. 15 000 tons of GOS in 1995[21]). The greater diversity of prebiotic oligosaccharides present because of sucrose could be advantageous for supporting the growth of a greater diversity of probiotic organisms compared to GOS produced from lactose alone.

In conclusion, the widely reported phenomenon of increased oligosaccharide yield at elevated substrate concentrations was investigated using Biolacta FN5 with lactose or a mixture of lactose and sucrose. It was found that decreases in water activity because of lactose at 40 °C had a minimal impact on GOS yield. Increases in GOS formation rate seem more important than decreases in GOS degradation rate for explaining the increased yields of GOS at higher lactose concentrations. It also seems that the transfer rate and yield from total saccharide were more sensitive to the lactose concentration than the combined concentration of lactose and sucrose.

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ABBREVIATIONS USED

GOS, galactosyl oligosaccharide(s); Lac, lactose; Suc, sucrose; Gal, galactose; Glc, glucose; Fru, fructose; HPLC, high-performance liquid chromatography; a_w, water activity; V_{max}^{app}, apparent maximum reaction velocity constant from the Michaelis−Menten equation; K_m^{app}, apparent Michaelis constant from the Michaelis−Menten equation

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