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Continuous production of oligosaccharides from whey using a membrane reactor

Mervat I. Foda [1], M. Lopez-Leiva *

Department of Food Engineering, Lund University, PO Box 124, S- 221 00, Lund, Sweden

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Abstract

Whey permeate (containing 14, 20 or 23% lactose) was used in the continuous production of oligosaccharides (OS) by hydrolysis from Maxilact 2000 L (*Kluyveromyces lactis* β-D-galacosidase). Two types of membrane reactors were used: a laboratory scale, Amicon stirred cell, using a flat membrane (41.8 cm^2) and a pilot plant-scale membrane reactor using a Romicon hollow fiber cartridge (0.5 m^2). Batch experiments were run to determine the optimal reaction time (when the largest amount of OS are produced). This optimal reaction time was then used in the experiments in continuous mode as the mean residence time of the whey permeate in the reactor, which could be controlled by adjusting the rate of permeate and or the volume of the substrate in the system.

Keywords: Oligosaccarides; Membrane reactor; Milk whey; Hydrolysis; UF-permeate

1. Introduction

World-wide production of whey is of the order of 130 million tonnes per year, with cheese production increasing at a yearly rate of about 3%. The pressure of anti-pollution regulations demands that the dairy industry develops new technologies that can change the role of whey from waste to a valuable product [1].

Lactose is the main component of the different types of whey (sweet, acid, whey-UF permeate) and it has been found that, during the enzymic hydrolysis of lactose, besides the main products of the hydrolysis (glucose and galactose), considerable amounts of oligosaccharides (OS) are also formed [2].

OS find particular use in promoting the growth of bifidobacteria in the lower part of the human intestine [3]. Lacto-oligosaccharides can be produced from whey permeate by enzymic hydrolysis using β-D-galactosidase. The enzyme can be used in two ways: in batch or in immobilised form. In the batch process, the enzyme, which is initially added to the batch, is lost at the end of the run when the hydolysate is pasteurised. If the enzyme is to be preserved, it can be immobilised by physical attachment to a carrier or it can be rendered immobilised by keeping it inside a UF-membrane reactor. This latter is the approach used in this work. Due to the difference in sizes between the enzyme and the solutes (glucose, galactose, oligosaccharides and non reacted lactose), the enzyme was kept in the ultrafiltration unit while the sugars (including the non reacted lactose) permeate the membrane and were collected outside the vessel.

The main objective of this work was to study the continuous partial hydrolysis of lactose to oligosaccharides and specifically the effect of enzyme and lactose concentration in oligosaccharide production.

2. Materials and methods

2.1. Whey

Whey solutions at different levels of lactose (14, 20 or 23%) were prepared from whey powder. The powder,

* Corresponding author. Tel.: +46-46-222-9814; fax: +46-46-222-4622.

E-mail address: miguel.lopez-leiva@livstek.lth.se (M. Lopez-Leiva)

[1] Present address: Food Technology and Dairying, National Research Centre, 3 Cairo, Egypt.

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(Arla Foods, Stockholm, Sweden, 80% lactose) was rehydrated with distilled water and stirred for 1 h at 40°C. A hollow fiber membrane (Romicon PM 10) with an effective area of 0.5 m^2 and cut off 10 000 was used to prepare the permeate. The solution was adjusted to pH 7 by adding NaOH.

2.2. Enzyme

Kluyveromyces lactis β-D-galactosidase (Maxilact® L 2000, Gist-Brocades NV, Delft, Holland), with molecular weight 135 000 Da was used in all experiments. Each gram contains 2000 neutral lactose units (NLU). One NLU is defined as that quantity of enzyme which will liberate 1.0 μmol *O*-nitrophenol per min under the conditions of the test.

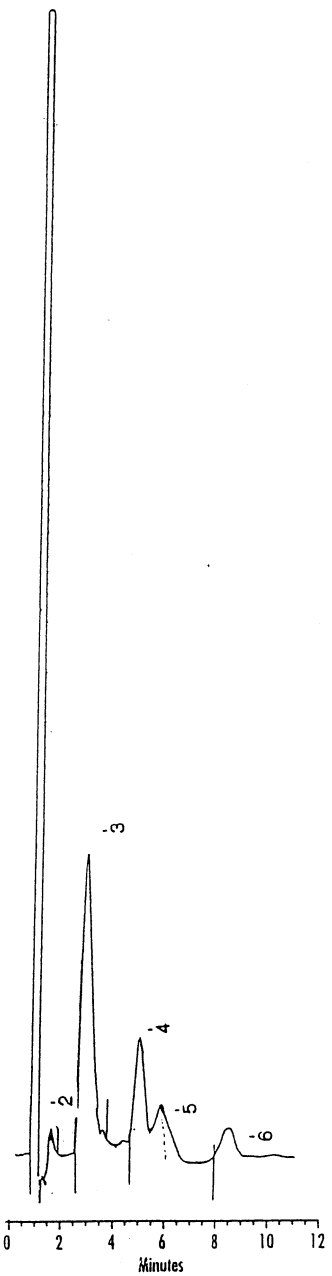

Fig. 1. HPLC chromatogram of hydrolysed whey permeate (initial lactose, 23%; enzyme, 0.1%; pH = 7; temperature = 45°C. (1) solvent, (2) unknown, (3) glucose plus galactose, (4) lactose, (5) and (6) oligosaccharides. Sample dilution factor: 5x.

2.3. Analytical methods

Glucose, galactose, lactose and oligosaccharides were determined by HPLC according to Jeon and Mantha [4]. The HPLC system consisted of a LCC 500 pump, a motor injector valve PMV-7 with a 20 μm loop, a Model ERC-7520 refractive index detector, a two channel recorder REC-482, and a carbohydrate analysis column (3.9 × 300 mm). The flow rate was maintained at 2.0 ml/min, and attenuation 4x. The mobile phase was prepared from acetonitrile (75%) and distilled water (25%) filtered through a sterile filter of 0.22 μ. The mixture was finally degassed for 30 min by ultrasound.

All analyses were made in triplicate.

Fig. 1 shows a chromatogram that illustrates the separation, by HPLC, of the sugars obtained from whey permeate hydrolysis. Two oligosaccharides consisting of three and four monosaccharides units were separated. Eventual di-oligosaccharides could not be distinguished from lactose by the HPLC column used. From the hydrolysis of lactose by Maxilact 40 000 and using gel chromatography, other researchers have identified six different oligosaccharides (di-, tri- and tetrasaccharides) [5].

2.4. Experiments in batch

In order to determine the optimal reaction time (when the largest amount of oligosaccharides was produced) eight batch experiments were performed. One hundred millilitres of whey permeate (14% or 23% lactose) were incubated with 0.05 or 0.1% (v/v) Maxilact L 2000 at 35 or 45°C in a water bath for a total time of 5.5 h. Samples were withdrawn at 30 min intervals, immersed in a boiling water bath for 5 min to inactivate the enzyme and analysed for sugars.

The results of these experiments showed that the optimal reaction time for all the cases was around 4 h. Accordingly, the conditions of the experiments in continuous were set to give an average residence time of the lactose in the reactor (= reaction time) of ≈ 4 h.

2.5. Experiments in continuous

2.5.1. Laboratory scale experiments

Continuous production of oligosaccharides from whey permeate in laboratory scale, was performed in the equipment schematically shown in Fig. 2.

In one experiment, 300 ml of whey permeate, containing 23% lactose were incubated with Maxilact L

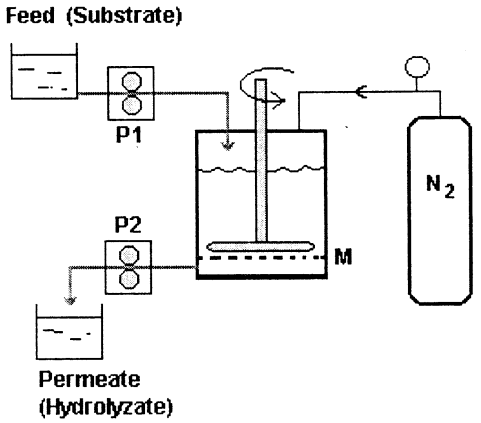

Fig. 2. Continuous, laboratory scale, experimental set-up. N_2: Nitrogen tube; M: UF membrane; P1, P2: peristaltic pumps.

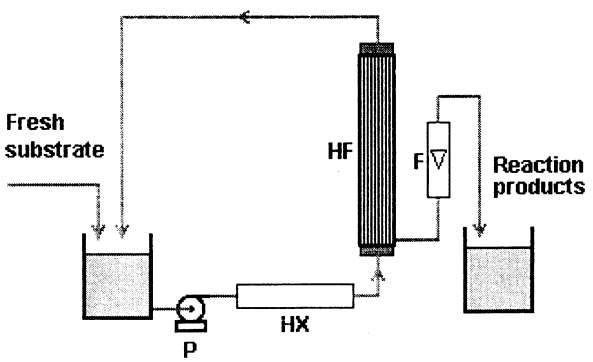

Fig. 3. Continuous, pilot plant scale, experimental set up. HF: hollow fiber cartridge; F: flowmeter; HX: heat exchanger; P: pump.

2000 (0.1% v/v) in a water bath for 4 h, at 45°C. The substrate (whey permeate and enzyme) was poured into the stirred ultrafiltration cell (Amicon, model 8400, 41.8 cm^2 effective membrane area), having a UF membrane (UF-CA-100 / PET 100, Hoechst AG, Germany). The volumes of the fresh feed (whey permeate) and the reaction products (permeate from the membrane reactor) were kept constant by means of the peristaltic pumps P1 and P2. These pumps are set in order to give a flow rate through the membrane reactor of about 1.2 ml/min at a pressure of 1.5 bar. This is the value needed to obtain a mean residence time of the substrate in the reactor of around 4 h. The temperature was kept at 45°C using a water bath. Samples were taken every 15 min until the end of the experiment (2.75 h).

Under these conditions a permeate flux of around 15 l/h per m^2 is obtained. A commercially sound permeate flux should be at least three times higher, but in this case this value gives an opportunity for monitoring the constancy of the experiments.

2.5.2. Pilot plant experiments

Continuous production of oligosaccharides from whey permeate, in pilot plant scale, was performed in a process set up as the one schematically shown in Fig. 3.

The membrane reactor consisted of a UF-hollow fiber Romicon module (PM 10), with nominal molecular weight cut off 10 000, and an effective area of 0.5 m^2.

In one experiment 11 l of whey permeate containing 20% lactose were incubated with 0.5% (v/v) Maxilact L 2000 in a water bath at 45°C for 4 h. Then the substrate (whey permeate and enzyme) was poured in the feed tank and recirculated through the hollow fiber UF module. The reaction products (glucose, galactose, oligosaccharides and non-hydrolysed lactose) permeated through the membrane. The volume of solution in the reactor was kept constant by adding fresh feed to the reactor. The temperature was kept constant at 45°C by means of a heat exchanger and the pressure of the system was monitored in order to obtain the desired residence time in the reactor (4 h). For a substrate volume of 11 l, a throughout flow of 2.75 l/h was necessary, which, for a membrane area of 0.5m^2, gave a permeate flux of around 5.5 l/h per m^2. As in the previous section this flux was far from being of commercial interest, but it makes easy the control of the experiments. In an industrial application this ultrafiltration equipment can probably handle a permeate flow (our product) ten times higher (25–30 l/h) for which a substrate volume of around 100 l is needed.

3. Results and discussion

3.1. Batch experiments

Eight batch experiments were conducted where the hydrolysis of lactose and the production of oligosaccharides were monitored along the reaction time (total 5.5 h). Maxilact (concentrations 0.05 and 0.10%) was used in all the experiments. Two initial lactose concentrations (14 and 23%) were tested as well as two temperatures (35 and 45°C) and two pHs (5 and 7). Fig. 4 shows the result for the following conditions: Maxilact 0.10%, 23% initial sugars, $T = 45°C$ and $pH = 7$

Similar figures were obtained for all the conditions and the numerical results, indicating the optimal time (when largest amount of OS are formed) and the concentration of OS obtained are found in Table 1.

The optimal time was around 4–4.5 h in all the cases and the maximum OS concentrations obtained varied between 1.9 and 5.1%.

As seen from Fig. 4 (the same situation is found in all the other cases), the oligosaccharides produced go through a maximum concentration to decrease afterwards indicating that they re-hydrolyse at larger incubation times. Burvall et al. [6] found that the oligosaccharides formed during the hydrolysis of lac-

tose by Maxilact 40 000, were not re-hydrolysed by prolonged incubation times (up to 14 days), but other researchers [2,7] have found a similar behaviour.

3.2. Continuous hydrolysis in a laboratory scale membrane reactor

An example of the results obtained in the continuous production of oligosaccharides when using a stirred UF-cell (Amicon) as a membrane reactor, is shown in Fig. 5. The conditions of this experiment were: initial lactose concentration, 23%; enzyme concentration, 0.1%; pH = 7 and temperature, 45°C. After an incubation time of 4 h, where the hydrolysis was allowed to proceed without extracting any product, the reactor was pressurised and the conditions of the experiment set in order to produce a residence time (equal to reaction time) = 4 h. New substrate was fed to the reactor continuously at a rate equal to the product being withdrawn. An OS concentration of around 4.5% was kept more or less stable during the continuous phase (2.75 h).

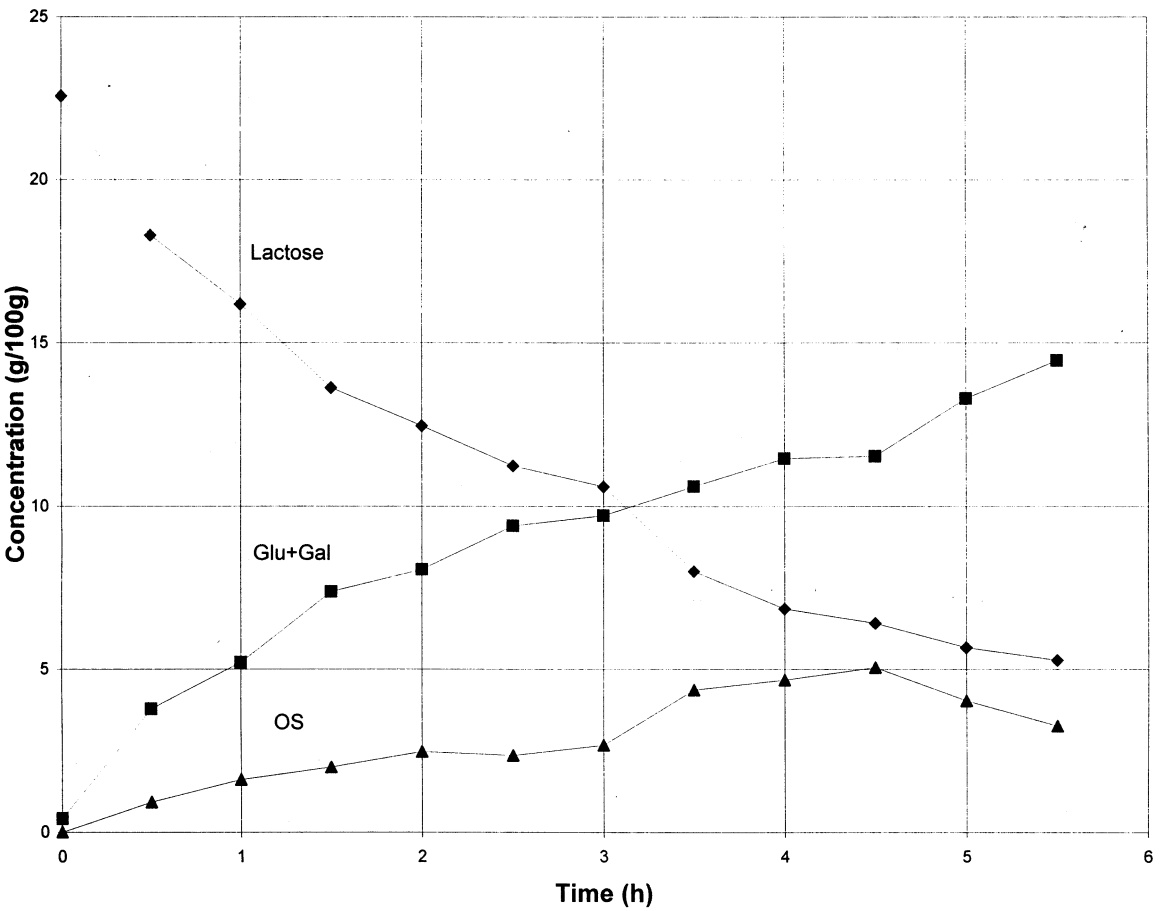

Fig. 4. Production of oligosaccharides in batch whey permeate. Initial lactose concentration, 23%; enzyme concentration, 0.1%; pH = 7; temperature = 45°C.

Table 1
Batch experiments, optimal reaction time and corresponding maximum OS concentration

Initial lactose, %	Enzyme, %	Temperature, °C	pH	Optimal. time, h	Max OS concentration, %
14	0.05	35	5	4.5	1.9
14	0.10	45	5	4	2.6
14	0.05	35	7	4.5	2.6
14	0.10	45	7	4	2.3
23	0.05	45	5	4	3.0
23	0.10	35	5	3.5	2.8
23	0.05	35	7	4.5	3.1
23	0.10	45	7	4.5	5.1

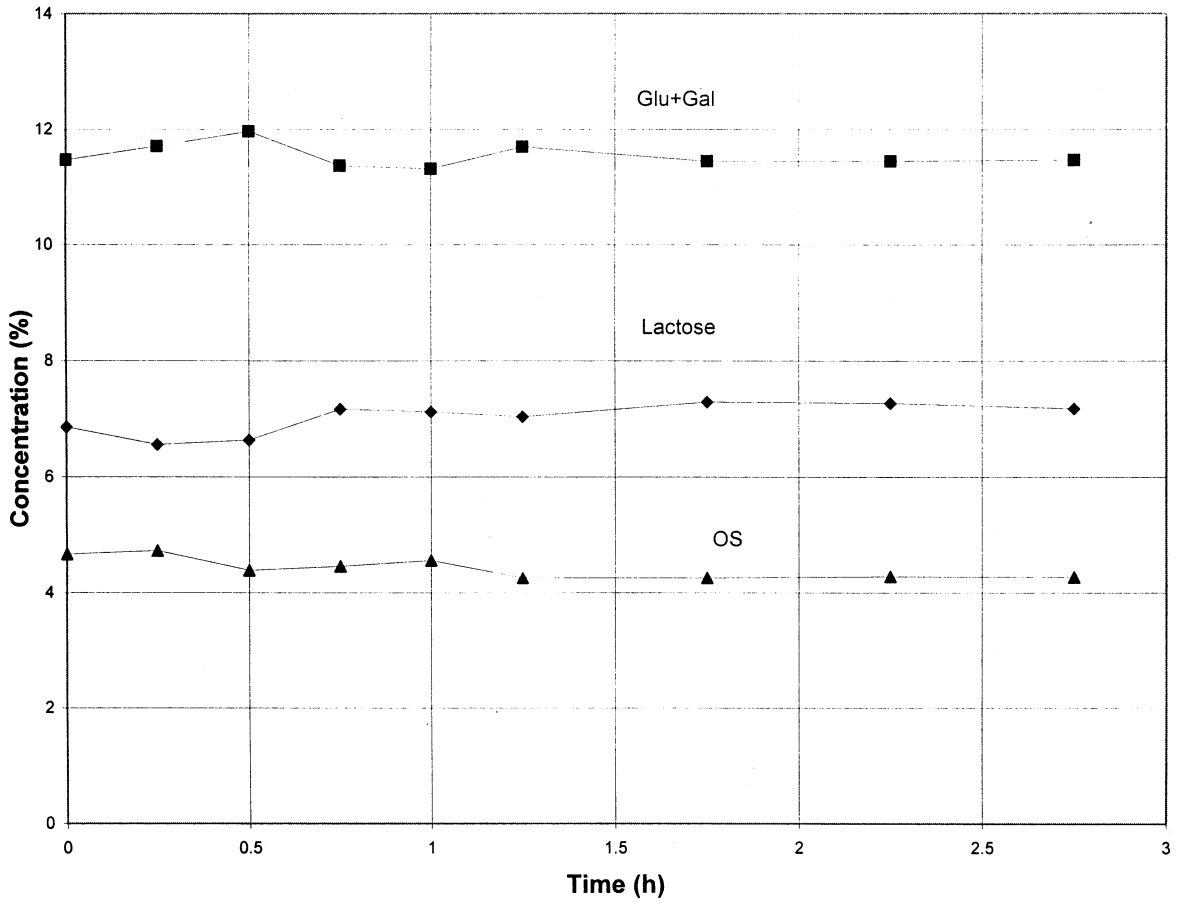

Fig. 5. Continuous production of oligosaccharides using a laboratory scale membrane reactor. Whey permeate, initial lactose concentration, 23%; enzyme concentration, 0.1%, pH = 7, temperature = 45°C.

Lactose concentration was also constant during all the continuous phase and equal to the concentration reached after the period of incubation. The yield of oligosaccharides (concentration of oligosaccharides /initial lactose concentration) was 20% with 70% DH.

3.3. Continuous hydrolysis in a pilot plant membrane reactor

Three experiments (pH = 7 and T = 45°C) were run at two concentrations of lactose (14 and 20%) and two concentrations of the enzyme (0.05 and 0.5%).

An example of the results obtained is shown in Fig. 6. The conditions of this experiment were: initial lactose concentration, 14%; enzyme concentration, 0.01%; pH = 7 and temperature, 45°C.

As in the previous case, before starting the experiments, the substrate was hydrolysed in batch mode during a period of 4 h. After this the partially hydrolysed substrate was fed to the reactor and the pump was started. From this moment permeate began to be withdrawn and the volume in the reactor was held constant by adding fresh feed.

The operational parameters of the process (flowrate and total volume of substrate) were set as to obtain a constant reaction time ≈ 4 h.

In Table 2 the results of the experiments are summarised, showing the concentration of oligosaccharides obtained as well as the yield and degree of hydrolysis.

The degree of hydrolysis decreased with increasing initial lactose concentration.

These results are in agreement with those of Huffman and Harper [8,9] who found that the degree of hydrolysis decreased from 80 to 35% when the initial lactose concentration increased from 4.5 to 20%. This result was obtained after 4 h of incubation with 1.0 g/l *A. Oryzae*-β-galactosidase using a UF hollow fiber membrane reactor at 50°C.

4. Conclusions

Oligosaccharides can be produced continuously using a membrane reactor. The optimum conditions of operation can be obtained from previously run batch experiments. According to these results, and for maximum oligosaccharide production, the residence time of the lactose in the reactor has to be ≈ 4 h.

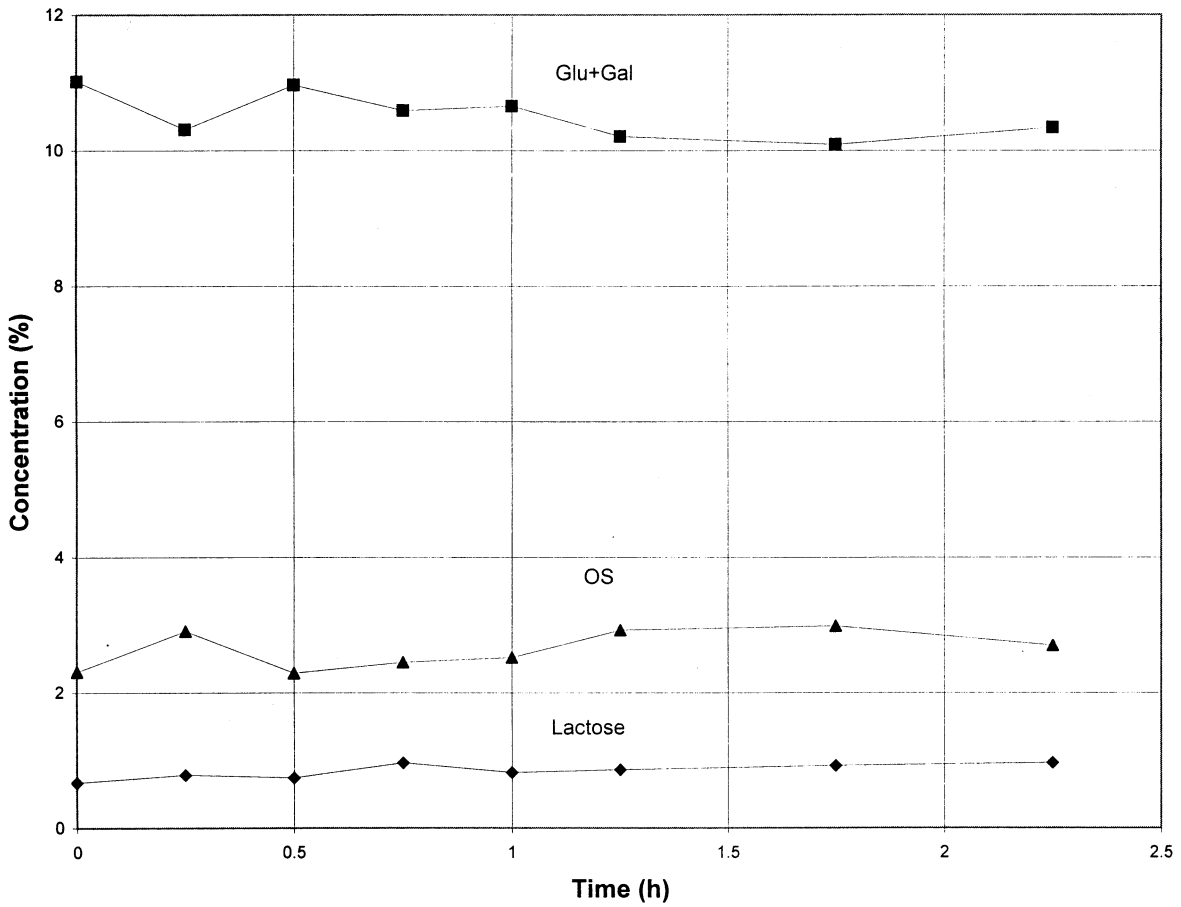

Fig. 6. Production of oligosaccharides using a pilot plant scale membrane reactor Whey permeate, initial lactose concentration, 20%; enzyme concentration, 0.5%; pH = 7; temperature = 45°C.

Table 2
Continuous OS production in a pilot plant membrane reactor

Initial lactose concentration (%)	Initial enzyme concentration (%)	OS concentration (%)	OS yield (%)	Degree of hydrolysis (%)
20	0.50	6.2	31	87.0
20	0.05	4.4	22	66.7
14	0.05	2.6	13	92.9

The Amicon membrane reactor (laboratory scale) keeps maximum yield of oligosaccharides ($\approx 20\%$), continuously for 2.45 h.

When using a pilot plant scale UF-hollow fiber membrane reactor, the largest yield of oligosaccharides obtained was 31% for a whey UF permeate containing initially 20% lactose and 0.5% Maxilact.

It is reasonable to assume that larger concentrations of both initial lactose and enzyme will produce larger amounts of oligosaccharides, but a better way of obtaining larger concentrations of OS will be to use membrane technology (nanofiltration) in order to concentrate the OS from the mono- and disaccharides. This allows the possibility of a continuous system where these two latter sugars can be re-feeded into the membrane reactor.

For further research it will be necessary to improve the HPLC analysis of the sugars in order to distinguish di-oligosaccharides (Glu-Glu and Gal-Gal) from lactose (Glu-Gal).

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