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Continuous Recycle Enzymatic Membrane Reactor System for *In-situ* Production of Pure and Sterile Glucose Solution

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Abstract: In this study, an efficient Continuous Recycle Enzymatic Membrane Reactor (CREMR) system for production of *in-situ* glucose solution was developed and the Simultaneous Gelatinization, Liquefaction and Saccharification (SGLS) carried out at temperatures below 60°C, is proposed to replace the conventional starch hydrolysis. Using a 30 kD polysulfone hollow fibre membrane and 10% (w/w) tapioca starch concentration, it is found that during the steady state continuous operation, the SGLS process in the CREMR at temperatures of 55 and 60°C and trans-membrane pressures of 0.5 and 1 bar has produced a steady state glucose concentration in the permeate stream as high as 64 g L^{-1} over a period of eight hours operation. The glucose solution obtained is of high purity greater than 99.9% and sterile, hence can be utilised as intravenous dripping solution and other medical products without post-treatments. In addition, the CREMR system is also relatively easy to scale-up, has a smaller footprint c.f. conventional systems, thus allowing *in-situ* production.

Key words: Glucose production, starch hydrolysis, continuous recycle enzymatic membrane reactor, amylase enzymes

INTRODUCTION

Sterile and pure glucose solution is being used in a large quantity in medical, pharmaceutical and biotechnology applications such as intravenous drips, pure culture of cells and tissues and ingredients for drug suspension. The industry that producing biopolymers such as xanthan gum and polylactic acid also uses pure glucose as a substrate as it gives high yields as compared to other carbon sources (Sarbatly and England, 2004, 2006) . Therefore, there is a high demand to develop a practical and reliable process that can produce *in-situ* and continuous pure and sterile glucose solution to be used in these applications.

Paolucci-Jeanjean *et al*. (2000a; 2000b; 2000c and 2001) have attempted hydrolysing the tapioca starch in the Continuous Recycle Membrane Reactor (CRMR). In principle, they have successfully produced a mixture of low Degree of Polymerisation (DP) of saccharides as permeate. Despite that, high reaction temperature at 80°C increased the initial solute viscosity, due to high starch gelatinisation rate. In concept, the relative optimum temperature of the slowest rate of reaction (saccharification) out of the three reactions (gelatinisation, liquefaction and saccharification (SGLS)) should be selected, thus the gelatinised starch will instantly be converted to glucose. To support the possibility of the SGLS proposal, two research groups, Marchal *et al.* (1999) and Linko and Javanainen (1996), investigated that the glucose can gradually be produced by enzymatic hydrolysis at low reaction temperature below 60°C in the batch reactor.

In this study we investigated the feasibility of the Continuous Recycle Enzymatic Membrane Reactor (CREMR) system for *in-situ* glucose production. A low temperature simultaneous gelatinisation, liquefaction and gelatinisation reactions (SGLS) reactions is also proposed.

MATERIALS AND METHODS

Materials: Tapioca starch powder was purchased from Thai World Import and Export Co., Ltd and the α-amylase enzyme (EC3.2.1.1) extracted from *Bacillus licheniformis* sp. and amyloglocosidase enzyme (AMG) (EC 3.2.1.3) extracted from *Aspergillus niger* sp. were purchased from Novo Nordisk. The polysulfone hollow fibre membrane cartridge model HF 1.0-43-PM30, serial number 4P3x 673 1 supplied by Romicon USA was used. The lumen diameter was 1.0922×10^{-3} meter and the total membrane area was 0.1 m^2. A 5 kD of the ceramic

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membrane supplied by Orelis company, France with a length of 0.035 m, 3.5×10^{-3} m lumen diameter, 19 channel and 0.0816 m^2 membrane area was also used for enzymes isolation.

Rejection of enzymes: An equal concentration mixture of termamyl and AMG was prepared at 0.6 mL in a two litre bottle. The enzyme solution was pumped into a 30 kD polysulfone hollow fibre membrane at room temperature (20°C), one bar pressure and 15 mL cross flow rate. The retentate stream was recycled back into the bottle while the permeate stream was weighted and samples were collected periodically. When half volume of the enzyme solution has been filtered the experiment was stopped. The initial and final state of retentate and permeate streams were scanned using a Shimatzu uv-visible spectrophotometer (UV-VIS) at 200-400 nm. Purified water by reverse osmosis (RO) was used for blank experiment (base line). The enzyme solutions were pumped separately into the membrane module using the same procedure as mentioned above and a specific wavelength of 265 nm was used to determine the concentration of the enzymes. The enzymes rejection was also tested using a 5 kD ceramic membrane supplied by Orelis company. In this research, the termamyl and AMG solutions were prepared at 0.3 mL in two litre bottles, respectively.

The CREMR and its operation: Figure 1 shows the flow diagram of the CREMR used in this study. The system comprises of a mixing tank, reaction tank, settling tank, hollow fibre membrane cartridge and storage tank. The capacity of the CREMR excluding the mixing and storage tanks is 12.2 L. The technical specification of each unit operation is given in Table 1.

A feed of 10% (w/w) of tapioca starch was prepared in a mixing tank at ambient temperature. For this, 50 mg of the calcium chloride was added for every litre of water to enhance enzymes stability. Well-mixed tapioca starch solution was pumped into a reaction tank through a heat exchanger to pre-heat tapioca starch solution to 55 and 60°C. The reactor has a stirrer and was kept at 350 rpm. The reaction temperature was controlled at 55 and 60°C and an overflow pipe controlled the level. The product from the reactor entered the settling tank through an overflow pipe. The top product was pumped into the membrane reactor at 15 mL while the bottom product was recycled to the reactor at the flow rate of 0.4 mL. The settling was equipped with a level controller.

In the membrane reactor, the operating pressure was controlled at 0.5 and 1 bar. During the initial state, both permeate and retentate streams were recycled back into the reactor. When the solution reached a steady state temperature, 3.66 mL of α-amylase and 3.66 mL of AMG were added into the reactor. The permeate was also recycled back into the reactor for 15 min after the enzymes were added to produce homogenous solution along the system, then the permeate stream was switched to the storage tank for continuous glucose production. Fluxes and glucose solution samples were measured and monitored periodically. In the case of batch process, the permeate was recycled back into the reactor from the membrane reactor.

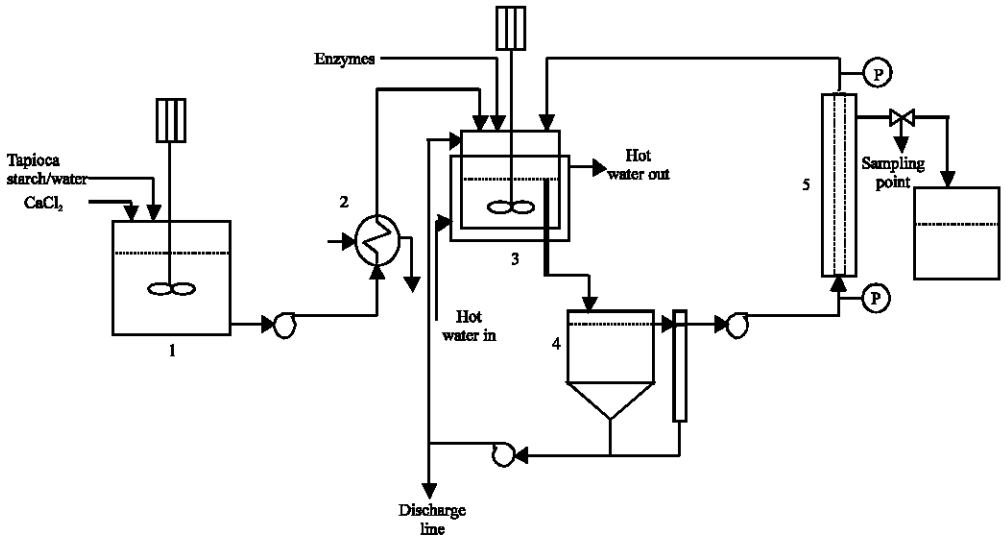

Fig. 1: The flow diagram of CREMR system. (1. Mixing tank, 2. Pre-heater, 3. Reactor, 4. Settling tank, 5. Membrane reactor, 6. Storage tank, p = Pressure gauge)

Table 1: Technical specifications of the physical unit operations of the CREMR

Operation	Specifications
Mixing tank	Max. operating capacity = 25 L, T = ambient, Stirrer and a Watson-Marlow 501U feed pump
Preheating	Water bath T~70°C, a stainless steel spiral coil with ϕ_i = 6.5 and ϕ_o = 3.5 mm. Spiral diameter ϕ = 185 and number of spiral is 4
Reactor	A thermo couple, vessel was jacketed by hot water to maintain the temperature, two buffers, a heidolph stirrer model RZR 2021, impeller and an overflow pipe with ϕ_i = 7.5 mm. Max. operating volume=5litres
Settling tank	A thermocouple, a float level controller, two Millipore peristaltic pumps (capacity 6-600 rpm) for recycling and supernatant pumps, a transparent cylinder column mounted at the settling tank with ϕ_i = 46 mm and high = 450 mm, supernatant intake pipe ϕ_i = 3.5 mm and thermocouple. Max. volume = 7 L
Membrane reactor	One retentate valve, two regeneration valves, one regeneration pump, one pressure relieve valve (3 bar max), three pressure transducers in-out and permeate streams and one pressure gauge at retentate stream. A 30 kD polysulfone hollow fibre cartridge model no HF 1.0-43-PM30 and serial number 4P3x 673 1, with 1.0922×10^{-3} meter lumen and 0.1 m^2 total surface area. Membrane module was supplied by Romicon USA

Determination of the glucose concentration: Samples collected from the permeate stream were diluted to 1:10 ratio with distilled water and then they were filtered through a 0.45μm nylon filter using a syringe into vials. A Shimadzu HPLC with a refractometer index detector (RID-10A) and Agilent ZORBAX sodium Column with a guard were used for determining the glucose concentration. The column's temperature was maintained at 80°C. The eluent was 0.05% sodium azide in RO water and the flow rate was 0.3 mL.

Membrane regeneration: The membrane module was rinsed with hot tap water (50°C) in a back flow direction with 30 litres/hr flow rate for two h. Pure water tests were carried out before and after the experiment. Results indicated that greater than 98% of the flux was recovered.

RESULTS AND DISCUSSION

Rejection of enzymes: This research was to investigate the rejection of the active amylase enzymes with molecular weights varying from 10 kD to 210 kD (Gupta *et al*., 2003) using a 30 kD hollow fibre polysulfone membrane and a 5 kD ceramic membrane, therefore, allowing a comparison to be made with the results reported by Paolucci-Jeanjean *et al*. (200b). They mentioned that the Carbosep M4 membranes supplied by Orelis, France, with the molecular weight cut-off 50 kD rejected the termamyl enzyme and non-hydrolysed starch but allowed to the hydrolysates. Gan *et al*. (2002) was also reported that an Amicon PM10 with 10kD molecular weight cut-off permitted a total rejection of the cellulose enzymes and zero rejection of the reducing sugar.

Table 2 shows the percentage of the spectrum's transmittance at 265 nm with uv-light of the initial amylase enzyme solution, the final retentate and permeate. Since 75% transmittance in the permeate was recorded, it indicates that some amylase enzymes were passing through the 30 kD hollow fibre membrane. Therefore, a smaller molecular-weight cut-off membrane was proposed.

Table 3 shows the enzyme concentrations obtained in the permeate with the 5 kD ceramic membrane. The result also showed that 10% termamyl enzyme and 16% AMG were permitted. These two enzymes permeation results along with the result reported by Paolucci-Jeanjean *et al*. (2000b) and Gan *et al*. (2002) suggest that the presence of polysaccharides inhibit the passage of amylase enzymes, thus, in this work the hollow fibre membrane with 30 kD molecular weight cut-off was used.

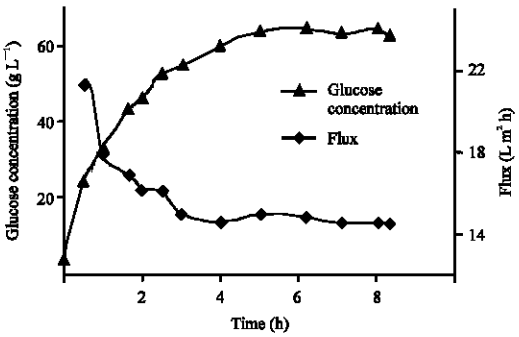

Fig. 2: Plots of the glucose concentration and flux at T_R = 55°C, T_M = 50°C and p = 0.5 bar

Table 2: Percentage of transmitted wavelengths of samples when using 30kD hollow fibre membrane

Samples	Wavelength (265nm) transmittance (%)
Initial	65
Retentate	64
Permeate	75

Operating conditions: Operating at room temperature, pressure one bar

Table 3: Enzyme passing through a 5kD ceramic Membrane

	Termamyl	AMG
Initial Concentration (ml enzymes /litre)	0.30	0.30
Permeate (ml enzyme /litre)	0.03	0.05
Percentage of the released enzyme (%)	10.00	16.00

Operating conditions: Operating at room temperature, pressure one bar

Glucose kinetics, fluxes and purity: Figure 2 shows the kinetic of glucose concentration and the flux at 55°C, 0.5 bar pressure and 10% (w/w) of tapioca starch. As can be seen, the glucose kinetic increased to reach to a steady state concentration at 64 g L^{-1} after 5 h operation. The flux was initially high at 21 litre/m^2 h and then decreased to a base line at 4.5 litre/m^2 h after three hours operation, which are most probably due to the concentration

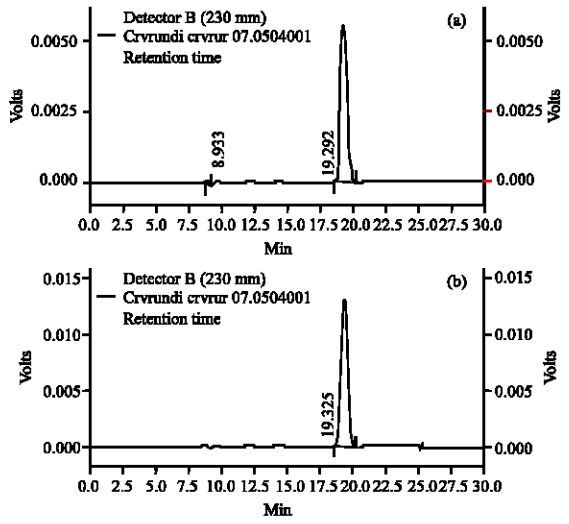

Fig. 3: The HPLC spectrum (a) after 0.5h operation (b) when steady state, after 5h. T_R = 55°C, p = 0.5 bar

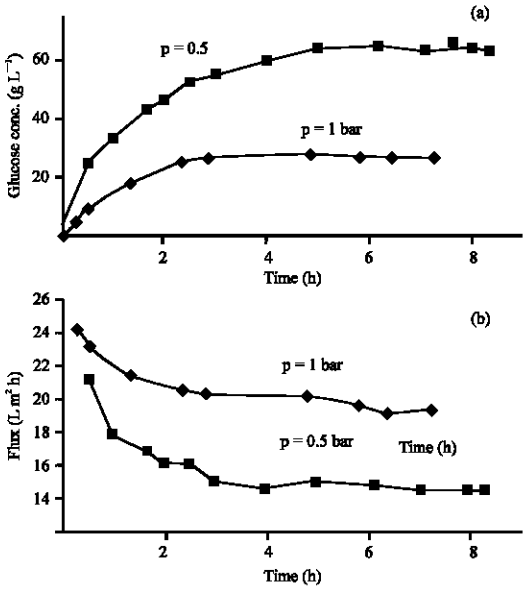

Fig. 4: Plots of (a) kinetics of the glucose production and (b) fluxes, at T = 55°C, $\sigma_{E^{mix}_0}$ = 0.6 mL and γ_{S_0} = 100 g L^{-1}

polarization effect and the increase of glucose concentration over time increases the dynamic viscosity of solute.

Figrue 3(a) and (b) show the polysaccharides spectrums in permeate stream after 0.5 and 5 0.5 bar. As can be seen in Fig. 3(a), the highest peak obtained at 9.23 min h continuous operation of the CREMR system with 10% (w/w) starch, at 55°C and represents greater than 99% purity of glucose at 24 g L^{-1} concentration. After 5 h operation the glucose purity remained high at greater than 99.9% and the glucose concentration increased to 64 g L^{-1} as shawn in Fig 3(b). These results suggest that the SGLS carried out in the CREMR system using a 30kD polysulfone membrane can produce pure glucose solution and effectively retaining the other polysaccharides.

Effect of operating membrane pressure: Figure 4 (a) shows the kinetics of the glucose production for both pressures of 0.5 and 1 bar. As can be seen, when one bar and reaction temperature at 55°C was used the glucose concentration was reduced by 2.5 fold compared to 0.5 bar. In Fig. 4(b), the flux increased by 35% when the pressure was increased from 0.5 to 1 bar, hence it is evident that the applied pressure can be used for controlling the glucose concentration in the permeate stream. It also means that the viscosity of the mixture decreased at one bar pressure due to low concentration of glucose in the membrane pores.

Table 4 shows the effect of the applied pressure on the steady state flux, the Hydraulic Residence Time (HRT) and the steady state glucose concentration. Operating the

Table 4: The effect of the applied pressure on the glucose concentration and the flux

Pressure (Bar)	0.5 bar	1 bar
The steady state flux (litre/m^2-h)	14.5	19.5
The steady state HRT, (h)	12.0 h	6.2h
The steady state glucose concentration (g L^{-1})	64.0	25.0

Conditions: T_R = 55°C, = 100g L^{-1}, 0.6 mL

CREMR at one bar pressure produced a high steady state flux at 19.5 l/m^2-h but low glucose concentration at 25 g L^{-1}. When operating the CREMR at 0.5 bars pressure, the steady state flux was low at 4.5 l/m^2-h but produced a high steady state glucose concentration at 64 g L^{-1} thereby suggesting that low pressures would give better conversion.

Effect of the temperature: Figure 5 (a) and (b) show effect of temperature on the glucose concentration and the flux at reaction temperatures of 55 and 60°C and one bar pressure. As can be seen in Fig. 5 (a), when the reaction temperature was 60°C, the glucose concentration was higher in the first three hours than the reaction temperature at 55°C. But, in both cases the glucose concentration reached an effectively the same steady state value.

This result suggests that varying the reaction temperature would change the maximum reaction rate but the final conversion would remain the same. This observation also in agreement with the result reported by Linko and Javanainen (1996) and Paolucci-Jeanjean *et al.* (2000b). Besides, as can be seen in Fig. 5 (b), the flux at

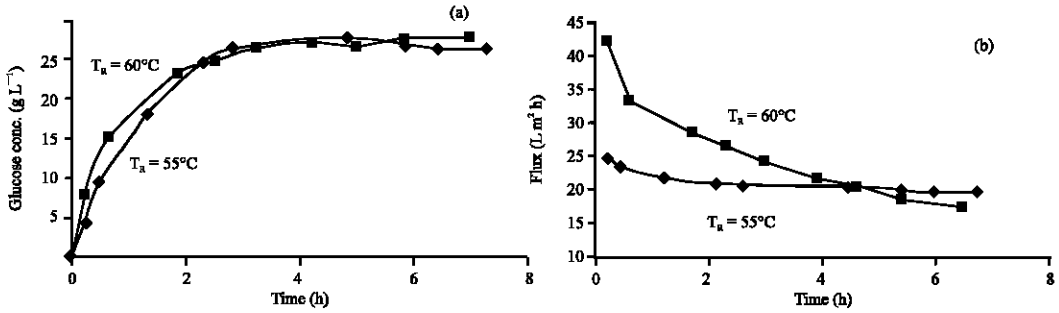

Fig. 5: Plots of (a) the glucose concentration and (b) the fluxes at P = 1bar $\sigma_{E^{mix}_0}$ ml/litre and γ_{S_0} g L^{-1} = 100g L^{-1}

reaction temperature of 60°C was initially higher than reaction temperature at 55°C and then it decreased to cross the flux curve of reaction temperature at 55°C after 5 h giving a lower flux thereafter.

As discuss earlier, this result is due to the increase in viscosity resulting in a low long-term flux. Moreover, this phenomenon can be explained as; (1) an imbalanced reaction between the gelatinisation and liquefaction reaction rates giving an increased of the solute viscosity, (2) the enzymes decay rate increases, (3) the membrane property changes with temperature and (4) the flux at reactor temperature at 60°C and at one bar has exceeded the critical flux.

Comparison of batch and continuous processes: The productivity of process is calculated based on the ratio between the weight of the produced glucose and the amount of enzymes for certain duration time as shown in 1;

$$\text{Productivity} = \frac{\sum W_{glucose}}{\phi \sum V_{E_0^{mix}}} \quad (1)$$

The $\sum W_{glucose}$ is the weight of glucose, $\sum V_{E_0^{mix}}$ the amount of enzymes and ϕ the operating time required. The weight of glucose in continuous operation is calculated by;

$$\sum W_{glucose} = V_R \cdot \gamma^{ss}_{glucose} + A_m \int_{t_0}^{\varphi} J_V \cdot \gamma_{glucose} dt \quad (2)$$

When the operation is relatively free from fouling,

$$\sum W_{glucose} = V_R \cdot \gamma^{ss}_{glucose} + A_m \int_{t_0}^{t_{ss}} J_V \cdot \gamma_{glucose} dt + A_m \cdot J^{ss}_V \cdot \gamma^{ss}_{glucose} (\varphi - t_{ss}) \quad (3)$$

The $\gamma^{ss}_{glucose}$, is the steady state glucose concentration, V_R the total volume of the CREMR, A_m membrane area, J_V the flux, t_{SS} the steady state time, J^{ss}_V the steady state flux. The productivity and the weight of glucose were calculated from the above Eq. 1-3 by using the Trapezoid method and the results are shown in Table 5. As can be seen from Table 5, in the batch mode operation, the conversion of tapioca starch is 69% compared to 62% in continuous process with the same enzyme quantity. Although less conversion in the continuous process was obtained, the production rate is higher than batch mode as calculated in-term of productivity. The productivity in continuous mode was increased three fold times than the batch operation. Moreover, 670 g of purified glucose was obtained while in batch, further product's purification is required.

Table 5: Comparison summary of batch and continuous processes carried out in the CREMR

	Batch	Continuous
Used tapioca starch	1.22 kg	2.32 kg
Purified glucose	0g	670g (61 g L^{-1})
Un-separated glucose	842 g (67g L^{-1})	781g (64 g L^{-1})
Weight of glucose	842 g	1450 g
Conversion of tapioca starch to glucose	69%	62%
Operating time	15 h	8.33 h
Used Enzymes	7.32 mL	7.32 mL
Productivity (g mL^{-1} h)	7.67	23.8

Conditions: T_R = 55°C, P = 0.5 bar, 100 g L^{-1}, = 0.6 mL

CONCLUSIONS

From the result obtained, it was found that the presence of polysaccharides has inhibited the amylase enzyme for complete rejection when the membrane with 30 kD mw cut-off is used. The applied pressure can also be used for controlling the glucose concentration in the permeate stream. The steady state concentration of glucose solution is also unaffected by the temperature but it does affect the maximum rate of reaction to attain fast steady state. The productivity in continuous process has been found to three times higher than that of the batch process. This work has demonstrated that it is possible to produce a pure and sterile glucose solution using the CREMR system using the SGLS operating strategy.

NOMENCLATURE

V	Filtrate volume (m^3),
A_m	Membrane area (m^2)
J_V	Flux (litre/m^2-h)
J_V^{SS}	Steady state flux (litre/m^2-h)
T_R	Reactor temperature (°C)
T_M	Membrane temperature (°C)
V_R	Volume of the CREMR (L)
$\sum V_{E_0^{mix}}$	Amount of enzymes (mL)
$\sum W_{glucose}$	Weight of glucose (g)
t_0	Initial time (h)
t_{SS}	Steady state time (h)

GREEK LETTERS

$\gamma_{P_{glucose}}$	Glucose concentration (g L^{-1})
$\gamma P^{max}_{glucose}$	Maximum glucose concentration, fixed 10% (w/v) starch concentration (g L^{-1})
γ_{S_0}	Initial substrate concentration (g L^{-1})
γ_S	Substrate concentration (g L^{-1})
$\gamma^{ss}_{glucose}$	Steady state glucose (g L^{-1})
$\sigma_{E^{mix}}$	Volume of the enzymes added over the total reactor volume occupied at 1:1 ratio of termamyl and AMG (mL).
ϕ	Operating time required obtaining weight of glucose (h)
ϕ_i	Coil diameter (mm)
	Internal diameter (mm)
ϕ_O	External diameter (mm)

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