System for automated kinetic characterization in a tube-in-tube reactor

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Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 October 2018 (18.10.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/189291 A1

(51) International Patent Classification:
B01J 19/00 (2006.01) *C12M 1/12* (2006.01)
B01J 19/24 (2006.01) *C12M 1/36* (2006.01)
C12M 1/40 (2006.01)

(21) International Application Number:
PCT/EP2018/059390

(22) International Filing Date:
12 April 2018 (12.04.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
17166205.9 12 April 2017 (12.04.2017) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: SYSTEM FOR AUTOMATED KINETIC CHARACTERIZATION IN A TUBE-IN-TUBE REACTOR

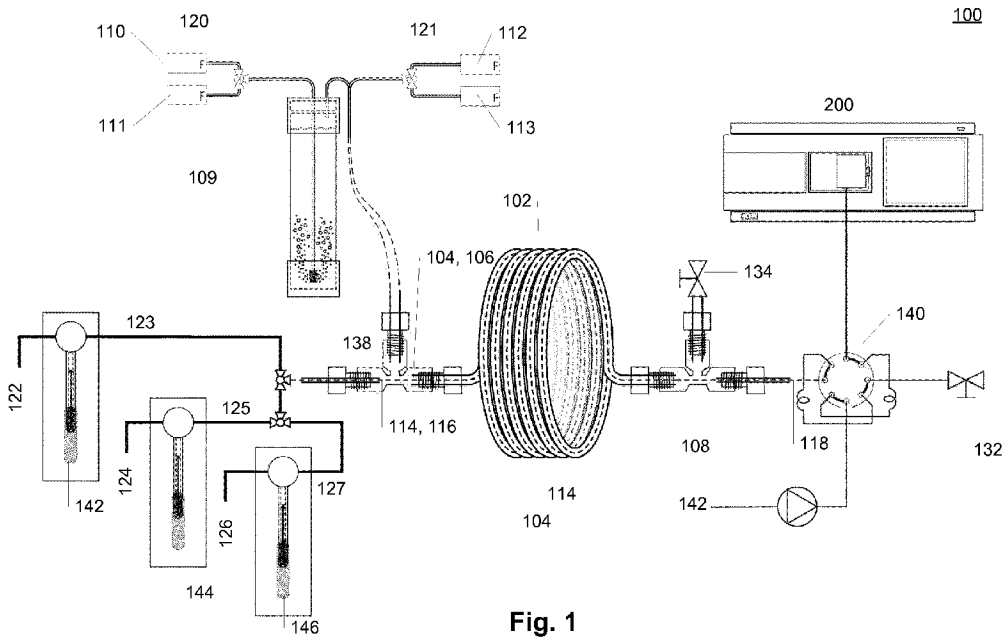

Fig. 1

(57) Abstract: Disclosed herein is a system for automated kinetic characterization in a tube-in-tube reactor, the system comprising at least one tube-in-tube reactor, one or more pumps for delivering the one or more liquids to the inner tube at or near the inlet end of the inner tube, one or more fluid delivering system for delivering the one or more fluids to the outer tube at or near the inlet end of the outer tube, and a spectrometric detector for measuring spectra of the liquid solution having passed through the tube-in-tube reactor, the spectrometer being positioned downstream of the tube-in-tube reactor.

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UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

System for automated kinetic characterization in a tube-in-tube reactor

The invention relates to a system for automated kinetic characterization in a tube-in-tube reactor.

Background

Enzyme or micro-organism-mediated reactions such as oxidation is of particular interest to synthetic organic chemists. However, the implementation of such systems demands knowledge of enzyme/micro-organism kinetics. Conventional wisdom holds that collecting kinetic data for e.g. biocatalytic oxidations is fraught with difficulties such as limited oxygen supply and low oxygen solubility in water.

As an example, selective oxidation is one of the most important transformations in synthetic organic chemistry. The importance of achieving high product purity in such transformations makes enzymes particularly interesting as potential catalysts on account of their exquisite selectivity in comparison with their chemo-catalytic counterparts. However, for process application it is often difficult to reach the required reaction intensity, e.g. reaction rate and product concentration. In particular, issues such as low enzymatic activity, product/substrate inhibition, co-factor regeneration and unfavourable thermodynamic equilibria need to be solved using biocatalytic reaction engineering. These problems are commonly investigated by studying the kinetic behaviour of an enzyme under different conditions.

Subsequently, using these data, challenges in reaching the required productivity can be addressed by either protein engineering or alternatively process engineering solutions to circumvent kinetic limitations. Regardless of the approach taken, enzyme improvement naturally starts in the hands of the protein engineer who typically screens for improved enzymes using single point measurements (i.e. at a single substrate concentration) to go through massive quantities of enzyme variants. In this way, protein engineering is frequently able to deliver improved enzymes capable of catalysing the conversion of non-natural substrates.

However, single point measurements only reveal apparent kinetics constants, such as the so-called specificity constant (V_{max}/K_M), which can be misleading as the basis for selecting the optimal enzyme. At points in development where selection is from a smaller pool of protein variants, it would be desirable to quantify the kinetics in full in order to have an adequate basis for deciding on the best enzyme for a given reaction, and reactor configuration. Moreover, it is necessary to determine the activity of an enzyme of interest

over the full range of potential operating conditions in order to truly assess the possibility of process implementation.

On this premise, we suggest that comprehensive kinetic investigations should be integrated into the improvement cycle of an enzyme. In this way it would be possible to direct screening to focus on evolving improved enzymatic kinetic properties, suitable for process implementation. While studying enzyme kinetics it is important to measure initial rates at substrate concentrations well above and below the true Michaelis constant(s) to determine these kinetic parameters with sufficient certainty. In the study of oxygen dependent enzymes, such investigations are notoriously difficult due to the limited solubility of oxygen in water, and to some extent the concomitant limited supply of oxygen.

The challenge of controlling the oxygen concentration leads in many cases to experiments being conducted at a single oxygen concentration (usually that in equilibrium with air, 276 µM). Air saturation is however insufficient to achieve enzyme saturation for several industrially relevant oxidases and it introduces uncertainty to parameter estimations. Indeed, conventional experiments can only reveal apparent Michaelis constants which are confined to the tested parameter space and should therefore be compared with great care.

Likewise, oxygen supply is often carried out by bubbling air through the reaction solution. However, in doing so, it is necessary to consider stripping of volatile substrate(s) and product(s) as well as potential enzyme deactivation at the gas-liquid interface.

Description of the invention

We present here a novel system and a novel method for the collection of kinetic data in enzyme or micro-organism mediated reactions using a pressurized tube-in-tube reactor, operated in the low-dispersed flow regime to generate time-series data. More specifically, disclosed herein is a system for automated kinetic characterization in a tube-in-tube reactor.

The system comprises at least one tube-in-tube reactor extending along a length from an inlet to an outlet end, the at least one tube-in-tube reactor comprising 1) an outer tube extending from an inlet end to an outlet end, wherein the outer tube is adapted for containing one or more fluids, and 2) an inner tube extending at least inside the outer tube from an inlet end to an outlet end, the inner tube having an inner diameter, wherein the inner tube is adapted for containing a liquid solution comprising one or more liquids.

By an inner tube extending at least inside the outer tube is meant that the inner tube may be longer than the outer tube.

The system may also comprise one or more pumps for delivering the one or more liquids to the inner tube at or near the inlet end of the inner tube, wherein the one or more pumps defines a flow rate of the one or more liquids in the inner tube.

Also included in the system is preferentially one or more fluid delivering system for delivering the one or more fluids to the outer tube at or near the inlet end of the outer tube.

The inner tube is normally non-porous or porous where all pores is having pore sizes below 300 nm in diameter. This means that effectively, the inner tube is without openings.

The one or more fluids may diffuse from the outer tube and into the inner tube. Also, the one or more fluids may be present in the inner tube and the outer tube at a saturation level, which is the same along a majority of the length of the tube-in-tube reactor. Thus, the saturation level may be differently over the length of the tube-in-tube reactor. By saturation level is meant that the fluid in the outer tube will contain a concentration of the transported compound which will saturate the fluid of the inner tube to a corresponding level.

The system additionally normally comprises a spectrometric detector for measuring spectra of the liquid solution having passed through the tube-in-tube reactor, the spectrometer being positioned downstream of the tube-in-tube reactor.

The system may additionally comprise an outer tube pressure regulator at the outlet end of the outer tube, and an inner tube pressure regulator at the outlet end of the inner tube. The pressure regulators normally control and ensure an equal or higher pressure inside the inner tube than inside the outer tube. By having an equal or higher pressure inside the inner tube than inside the outer tube it is ensured that the saturating component concentration is controlled by the content and pressure of the outer tube. For cases where the outer tubes fluid is a gas, the equal or higher pressure ensures that there is no gas phase formation in the inner tube.

The flow rate of the one or more liquids inside the inner tube and the inner provides a low dispersed flow of the one or more liquids with a Bodenstein number above 50. The flow rate is defined by specifics of the one or more pumps delivering the one or more liquids to the inner tube and the inner diameter of the inner tube. By “specifics” is meant that the liquid flow

is not measured as such, but instead is ensured to be within a specific range by choosing a well calibrated, precise fluid delivery system, such as e.g. a syrringe pump or pressure driven flow.

By low dispersion is meant a unique regime of laminar flow that occurs only at a microfluidic scale. In this flow regime, the radial mixing from the centre of the tube to the edges is governed purely by diffusion. At micro-scale, the diffusion lengths are by default very small and this will in turn give very short radial mixing times. Low dispersed flow will therefore flatten the well-known tongue profile of laminar flow and solute concentrations can thereby be assumed to only change along the length of the reactor. These dynamics makes it so that the reactor can be described by plug-flow behaviour.

By Bodenstein number (Bo) is meant a dimensionless number describing the ratio of convection to dispersion defined in the following form:

$$Bo = \frac{vL}{D_T} \qquad \text{Eq. 1}$$

where D_T is the Taylor dispersion coefficient, L is the reactor length (in this invention the tube-in-tube reactor length), and v is the average velocity of the one or more liquids.

By Taylor dispersion coefficient (D_T) is meant a number incorporating the effects of both diffusion (D) and convection defined in the following form:

$$D_T = D + \frac{v^2 d_t^2}{4\beta D} \qquad \text{Eq. 2}$$

where D is the diffusion coefficient of a solute (in this invention substrate, product or enzyme), d_t is the tube diameter, and β is a geometry parameter which is 48 for circular tubes. Convection dominates most small-scale flow systems, except when a system has an extremely small volumetric flow rate. As a result, the diffusive portion (D) of the Taylor dispersion coefficient (D_T) can be neglected bringing the Taylor dispersion coefficient to:

$$D_T \approx \frac{v^2 d_t^2}{4\beta D} \qquad \text{Eq. 3}$$

Inserting eq. 3 into Eq. 1 results in the following expression for the Bodenstein number (Bo):

$$Bo = \frac{L4\beta D}{v d_t^2} \quad \text{Eq. 4}$$

When having circular tubes, β is 48 (Levenspiel, O. Chemical Reaction Engineering, 3rd ed.; John Wiley and Sons: New York, 1999.). D ranges from 10^{-9} to 10^{-11} m^2/s. The average linear velocity in circular tubes is defined as:

$$v = \frac{Q}{A} = \frac{Q}{\pi \frac{d_t^2}{4}} \quad \text{Eq. 5}$$

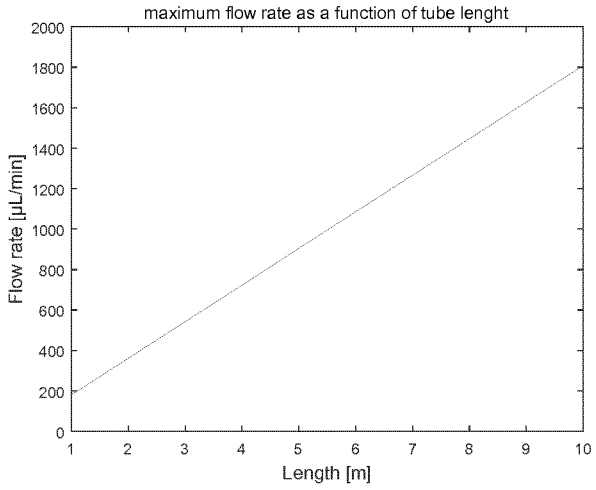
where Q is the volumetric flow rate and A is the cross sectional area of the tube. When inserting the expression for the average linear velocity into the expression for the Bodenstein number it yields:

$$Bo = \frac{L4\beta D}{\frac{Q}{\pi \frac{d_t^2}{4}} d_t^2} = \frac{L\pi\beta D}{Q} \quad \text{Eq.6}$$

Rearranging this equation in term provides an expression for the correlation between flow rate and the length of tube given by:

$$Q = \frac{L\pi\beta D}{Bo} \quad \text{Eq.7}$$

Inserting 50 as the Bodenstein number, Bo, and $1*10^{-9}$ m^2/s as the diffusion coefficient, the maximum flow rate as a function of the tube length can be plotted as shown below:



Disclosed herein in a second aspect of the invention is the use of a tube-in-tube reactor for collecting kinetic data of reactions catalysed by micro-organisms or enzymes, the reactions including:

- Oxidation reactions catalysed by enzymes such as oxidases, and dehydrogenases
- Hydrogenation reactions catalysed by enzymes such as hydrogenases;
- Amination reactions catalysed by enzymes such as transaminases; and
- Carboxylation reactions catalysed by enzymes such as carboxylases.

Further disclosed herein in a third aspect of the invention is a method for collecting kinetic data of reactions catalysed by micro-organisms or enzymes, the method comprising the steps of:

- providing a tube-in-tube reactor;
- operating the tube-in-tube reactor in a low dispersed flow regime
- supplying the tube-in-tube reactor with an enzyme solution or a micro-organism suspension, and
- generating time-series of spectroscopic data.

By the above system, biocatalytic systems can be investigated with minimal material consumption. So by use of the system and/or the method according to the invention not only the high accuracy of the kinetic data is obtainable, but also accurate evaluation of Michaelis constants for oxygen, $K_{MO,}$ which are higher than the concentration of oxygen in oxygen saturated solutions at ambient conditions. For the first time this paves the way to integrate kinetic data into the protein engineering cycle.

The use of a low dispersed flow is a unique regime of laminar flow that occurs only at a microfluidic scale. In this flow regime, the radial mixing from the centre of a tube to the edges is governed purely by diffusion. At micro-scale, the diffusion lengths are by default very small and this will in turn give very short radial mixing times. Low dispersed flow will therefore advantageously flatten the well-known tongue profile of laminar flow and solute concentrations will thereby only change along the length of the reactor. These dynamics mean that the tube-in-tube reactor can be described by plug-flow behaviour.

Thus, by using the above system and method, at low residence times, steady-state is obtained and the flowrate is subsequently ramped down using an instantaneous flow rate equation, as e.g. the one from Moore and Jensen [Moore *et al.* (2014), “Batch” kinetics in flow: online IR analysis and continuous control, Angewandte Chemie (International Ed. in

English), 53(2), 470–473]. This allows measurements of initial rates (i.e. concentration-time profiles) without the need for multiple steady states.

The previous challenges of controlling the oxygen concentration, e.g. bubbling air through a reaction solution in order to supply the solution with oxygen, which in turn results in the necessity to consider stripping of volatile substrate(s) and product(s) as well as potential enzyme deactivation at the gas-liquid interface, is avoided by the use of the above system and method, where fluids such as gases may be supplied to the reaction solution with the enzymes and/or micro-organisms via a membrane, creating a bubble-free aeration system.

The system and method according to the above further allows control over oxygen as a substrate in oxygen-dependent enzyme reactions, when oxygen is one of the one or more fluids delivered to the outer tube. Furthermore, the tube-in-tube reactor satisfies the requirement for negligible change in substrate concentration for measurement of initial rates, since oxygen can be supplied along the tube-in-tube reactor as it is consumed.

By the above system, an automated flow reactor system that can be used for rapid characterization of the kinetics of oxygen-dependent enzymes is obtained. Here the oxygen level can be controlled and high concentrations, which are up to 25-fold higher than that possible using only air at atmospheric conditions, are obtainable. Time-series data with an enzymatic catalyst despite its low diffusivity is obtainable with the system of this invention. Further, the obtained data is in good agreement with experiments conducted in a batch system. The system is capable of characterizing the kinetics of most enzymes within the oxidoreductase class EC 1, where oxidation often delivers changes to the UV-spectra and enables quantification of product development. The system and method is however not limited to oxygen-dependent enzymes, but can be used to study many other enzymes requiring gaseous substrates, such as hydrogen (hydrogenase), carbon dioxide (formate dehydrogenase) or methane (methane monooxygenase).

The system and method presented herein can be used to introduce kinetic characterization of oxidoreductases into the catalyst development cycle, where biocatalytic reaction engineering can be used to guide both process and protein engineering.

The system and method presented herein may advantageously be primed for automation as controllers, pumps and detectors of the system may be computer controlled. Algorithms may therefore be applied not only to run experiments but also to conduct iterative design of experiments, such as model based design of experiments. Such possibilities further increase

the likelihood that the system according to the invention will deliver reliable kinetic information about the investigated biocatalyst.

Normally, the Bodenstein number is above 100, or above 200, or above 300, or above 400, or above 500, or above 600. The upper limit of the Bodenstein number may in one or more embodiments be above 10000.

In one or more embodiments, the system only comprises one tube-in-tube reactor.

Alternatively, the system comprises a plurality of tube-in-tube reactors arranged in serial. An additional inlet could be connected in between reactors and would then be used to study cascade reactions or to add fresh catalyst or to adjust pH.

Yet alternatively, the system comprises a plurality of tube-in-tube reactors arranged in parallel. Additional inlets/outlets could be connected to each of the tube-in-tube reactors.

In one or more embodiments, the multiple of tube-in-tube reactors includes two tube-in-tube reactors, or three tube-in-tube reactors, or four tube-in-tube reactors, or five tube-in-tube reactors. This applies both to the multiple of tube-in-tube reactors arranged in series and in parallel.

In one or more embodiments, the outer and/or the inner pressure regulators are selected from the group of:

- a seat,
- a needle,
- a relief
- a control valve,
- a piece of tubing with a significant pressure drop at the flow rate,
- a water column.

The above given examples of pressure regulators are not to be seen as a limited group. Other examples may also be imagined.

The inner pressure regulator and/or the outer pressure regulator may be controlled by means of a computer or a digital circuit. By “digital circuit” is meant electronics that operate on digital signals. Digital electronic circuits are usually made from large assemblies of logic gates.

In one or more embodiments, the inner pressure regulator and/or the outer pressure regulator is actuated so that the pressure of the inner tube and/or the outer tube, respectively, is adjustable to such that different pressure set points is obtainable during operation. This enables a much wider range of oxygen concentration to be investigated, from 5% at atmospheric pressure to 100% at 10 barg, which translates to oxygen concentrations in the inner tube of 0.064 mM to 14.1 mM. Concentrations at pressures up to 100 barg can also be investigated. By “different pressure set points” is meant that the pressure of the inner and outer tube volume is set according to different setpoints.

In addition to the pressure regulators, the system may further comprise an outer tube pressure monitor at the outlet end of the outer tube, and an inner tube pressure monitor at the outlet end of the inner tube. The pressure monitors may e.g. being a manometer or a pressure transducer. An example of a manometer is a 0-25 bar or 0-15 bar manometer.

The one or more pumps may in one or more embodiments include a first pump for delivering a liquid solution comprising a first enzyme type to the inner tube. Alternatively, a liquid micro-organism type solution may be delivered to the inner tube by a first pump.

The one or more pumps may also include a second pump for delivering a substrate solution to the inner tube.

The one or more pumps may further include a third pump for delivering a buffer solution to the inner tube. Both the substrate and the buffer solutions may advantageously be mixed with the liquid solution comprising enzymes.

The one or more pumps used in the system may advantageously be single pumps for delivering the one or more liquids, such as e.g. the enzyme, the substrate and the buffer solutions, to the inner tube in controlled portions. The pumps may be regulated independently of each other to provide e.g. a larger volume of the buffer solution compared to the enzyme solution.

In one or more embodiments, the one or more pumps are syringe pumps. This allows for a controlled delivery of very small amounts of liquid to the inner tube.

The inner tube is normally made from an inner tube material having a high oxygen permeability and the outer tube is normally made from an outer tube material having a low

oxygen permeability being significantly lower than the oxygen permeability of the inner tube material. In an example, permeability of the outer tube material is at least 100 times lower than that of the inner tube.

The difference in oxygen permeability will allow oxygen in the outer tube to diffuse into the inner tube but not escape the outer tube. Thereby oxygen saturation in the inner tube is obtainable after providing oxygen to the outer tube. This also applies for other gases supplied to the outer tube for inner tube materials being permeable to these materials.

Put differently, the inner tube in the tube-in-tube reactor may have a significantly higher oxygen permeability than the outer tube. In one or more embodiments, the permeability of the inner tube is at least 100 times higher than that of the outer tube.

The saturation level of the one or more fluids may be lower at the inlet end than at the outlet end of the inner tube.

In one or more embodiments, the saturation level of the one or more fluid at the inlet end of the tubes is between 0-21% and at the outlet end of the tubes is between 50-100%, such as 80-100%, or 90-100%, or 95-100%, or 99-100%.

In one or more embodiments, the inner tube material is selected from the group of:

- Fluorinated polymers, such as amorphous copolymer of tetrafluroethylene (TFE), and 2,2-bis-triflouromethyl-4,5-diflouro-1,3dioxole (PDD);
- Perfluorosulfonate polymers;
- Silicone based polymers, such as polydimethylsiloxane (PDMS) and poly(1-trimethylsilyl-1-propyne) (PTMSP);
- Polyethylene (PE);
- Polypropylene (PP), and
- Polycarbonate.

Examples of amorphous copolymer of TFE and PDD are Teflon AF 1600 or Teflon AF 2400. The perfluorosulfonate polymers may be Nafion. PE, PP and polycarbonate are examples of porous materials suitable as the inner tube material.

In one or more embodiments, the inner tube material is a polytetrafluoroethylene, preferably an amorphous copolymer of TFE and PDD.

The outer tube material may be selected form the group of:

- Polytetrafluoroethylene (PTFE);
- Polyethylene terephthalate (PET);
- Polyvinylidene fluoride (PVDF);
- Ethylene ChloroTriFluoroEthylene (ECTFE);
- PolyEther Ether Ketone (PEEK);
- Steel including stainless steel and steel alloys.

Examples of stainless steel and steel alloys include Stainless steel 304, 316, 316L and 317, Hastelloy C, Alloy 276, Hastelloy C4, and UNS10276.

In one or more embodiments, the outer tube material is polytetrafluoroethylene, preferably a semi-crystalline polytetrafluoroethylene.

The one or more fluid delivering system for delivering the one or more fluids to the inner tube may advantageously be a mass flow apparatus or pumps, such as e.g. syringe pumps.

In one or more embodiments, the syringe pumps have a displacement resolution between 20 pico liter to 40 nano liter. The ratio between volume of the tube-in-tube reactor and the displacement resolution should be between 4000 to 2.600.000.

In one or more embodiments, the one or more fluids delivered to the outer tube are mixed in their full range before entering the outer tube. Thus if e.g. multiple gases are delivered to the outer tube, the gases are mixed before entering the outer tube.

By the term fluid is include both liquids and gases. Thus, in one or more embodiments, the one or more fluids are liquids, and in one or more embodiments, the one or more fluids are gases.

When the fluids are gases, the system may further comprise a humidifier for humidifying the one or more gases before the one or more gases enters the outer tube. The one or more gasses are wetted before entering the tube-in-tube reactor in order to avoid the stripping of water through the inner tube.

In one or more embodiments, the humidifier is adapted for introducing concentrations up to 100% humidity at all temperatures within the tube-in-tube reactor.

The system may further comprise one or more gas mass flow controllers for controlling the gas flow into the humidifier.

A larger number of gases may be used depending on the reaction, which is to be studied. In one or more embodiments, the one or more gases is selected from the list of Oxygen (O_2), Nitrogen (N_2), Ammonia (NH_3), Carbon monoxide (CO), Carbon dioxide (CO_2), Hydrogen (H_2), Methane (CH_3).

Additional gases includes: Ozone (O_3), Ethane (C_2H_6), Ethylene (C_2H_4), Propane (C_3H_8), Propene (C_3H_6), Butane (C_4H_{10}), Butylene (C_4H_8), Hydrogen sulphide (H_2S), Nitrogen oxides (NO and/or NO_2), Sulfur dioxide (SO_2), and Hydrogen cyanide (HCN).

In one or more embodiments, the one of the one or more gases is N_2 contained in a nitrogen receptacle.

In one or more embodiments, the one of the one or more gases is O_2 contained in an oxygen receptacle.

Nitrogen and oxygen may be delivered to the outer tube simultaneously during an experiment using the system of this invention.

In one or more embodiments, one or more mass flow controllers varies the oxygen amount in the range 0-100% O_2. This provides an absolute control of the oxygen level in the outer tube and in the inner tube as oxygen diffuse into the inner tube.

In one or more embodiments, the oxygen amount is in the range 20-100% O_2, or in the range 40-100% O_2, or in the range 60-100% O_2, or in the range 80-100% O_2.

The tube-in-tube reactor may have a length which is adjusted to give a steady flow for the one or more pumps. The tube-in-tube reactor may e.g. have a length between 1-10 meter, or 1-7 meter, or 1-5 meter, or 2-4 meter, or approximately 3 meter. These lengths will ensure that a steady flow is present in the tubes – in particular for the tube-in-tube reactors with lengths between 2-4 meters.

The inner tube may be 10-15 cm longer than outer tube, or alternatively be up to 50 cm longer than the outer tube. By having the inner tube being longer than the outer tube, it becomes easier to couple the inner tube to e.g. the spectrometric detector.

The inner tube has an inner diameter defining the opening for the liquid, the inner diameter of the inner tube being for example between 0.025-0.50 mm, or between 0.10-0.25 mm, or between 0.15-0.25 mm, or between 0.18-0.22 mm, or approximately 0.20 mm.

In one or more embodiments, the inner tube has an outer diameter being twice as large as the inner diameter of the inner tube.

In one or more embodiments, the inner tube has an outer diameter between 0.1-0.6 mm, or between 0.3-0.5 mm, or between 0.35-0.45 mm, or between 0.38-0.42 mm, or approximately 0.4 mm. The outer diameter will however always be larger than the inner diameter providing the inner tube with a wall.

In one or more embodiments, the inner tube has a wall thickness between 10-500 µm, such as 50-200 µm, or such as 50-100 µm.

The outer tube has an inner diameter defining the opening for the one or more fluids and the inner tube, the inner diameter of the outer tube being e.g. between 0.5-10.0 mm, or between 0.5-7.5 mm, or between 0.5-5.0 mm, or between 0.5-3.2 mm, or between 1.0-2.0 mm, or between 1.2-1.8 mm, or between 1.5-1.7 mm, or approximately 1.6 mm. An inner diameter of 3.2 mm corresponds to 1/8 inch.

In one or more embodiments, the outer tube has an outer diameter between 1.4-13.0 mm, or between 1.5-10.0 mm, or between 1.58-6.4 mm, or between 2.8-4.0 mm, or between 3.0-3.6 mm, or between 3.1-3.3 mm, or approximately 3.2 mm. An outer diameter of 1.58-6.4 mm corresponds to 1/16-1/2 inch. The outer diameter will always be larger than the inner diameter providing the outer tube with a wall.

The system may further comprise a heating/cooling device for controlling the temperature of the fluids before entering the tube-in-tube reactor. Likewise, the system may further comprise a tube heating/cooling device for controlling the temperature of the fluids in the tube-in-tube reactor. The heating/cooling devices may be water baths, oil baths, peltier elements, heating sleeves, electric heat tracing, fluid heat trancing, and/or resistance heating wire.

The outlet of the inner tube is connected to a detector by means of an injection valve. Thus, in one or more embodiments, the outlet end of the inner tube is connected to the

spectrometric detector by means of an injection valve. The injection valve may e.g. be a switch valve. By using a switch valve, samples are carried from the injection loop into the spectroscopic detector where the solution is subject to flow injection analysis. The injection valve may be controlled manually or electronically.

The injection valves may be 8-port actuated injection valve with two 5 µL injection loops. Alternatively, four ports with 100 nL could be used.

Alternatively, the spectrometric detector may be coupled to the inner tube in an in-line manner. The use of valves is thereby made redundant.

A number of different spectrometric detectors may be used either as the only detector or as individual detectors simultaneously measuring spectra which in combination provide additional and/or complementary information. In one or more embodiments, the spectrometric detector is selected from the group of:

- ultra violet-visible detector or spectrometer;
- mass spectrometers;
- Raman spectrometers,
- NMR spectrometers;
- florescence detectors or spectrometers and
- infrared spectrometers.

Spectra are normally measured continuously over time, which allows the user to follow the reaction as it occurs in real time.

In one or more embodiments, the spectrometric detector is an ultra violet-visible (UV-vis) detector or spectrometer. An example of a UV-vis detector is a UV/VIS diode array detector. The diode array detector may be coupled to a standard 14 µL flow cell with 1 cm path length.

Possibly, an inlet UV spectrum is also provided in the system. The first spectrum, which is measured at the outlet is almost equal to the starting point spectrum.

The tube-in-tube reactor may further comprise one or more additional inner tubes arranged in parallel inside the outer tube. Thus, there are two or more inner tubes inside the same outer tube. The inner tubes are normally arranged in parallel inside the outer tube with a minimum of physical contact between the inner tubes keeping the surfaces area of each or the inner tubes as large as possible. This ensures that diffusion of the one or more fluids into

the inner tubes is as even as possible. By having more than one inner tube inside the outer tube allows for the study of multiple enzymes and/or micro-organisms simultaneously – one type being delivered to each of the inner tubes.

In one or more embodiments, each of the multiple inner tubes inside the outer tube is connected to a spectrometric detector. This allows for the simultaneous study of more than one type of enzyme and/or micro-organism. Alternatively, the inner tubes may contain the same type of enzymes or micro-organisms, which may then simultaneously be studied using different spectrometric detectors.

The tube-in-tube reactor according to the above may also be used for collecting kinetic data of reactions catalysed by micro-organisms or enzymes. The reactions include – but are not limited to:

- Oxidation reactions catalysed by enzymes such as oxidases, and dehydrogenases
- Hydrogenation reactions catalysed by enzymes such as hydrogenases;
- Amination reactions catalysed by enzymes such as transaminases; and
- Carboxylation reactions catalysed by enzymes such as carboxylases.

The tube-in-tube reactor system according to the above may further be used in a method for collecting kinetic data of reactions catalysed by micro-organisms or enzymes. The method may comprise the steps of:

- providing a tube-in-tube reactor according to the above;
- operating the tube-in-tube reactor in a low dispersed flow regime;
- supplying the tube-in-tube reactor with an enzyme solution or a micro-organism suspension; and
- generating time-series of spectroscopic data.

In one or more embodiments, the micro-organisms or enzymes are gas dependent. The gas dependent enzyme may be selected from the group of:

- Hydrogenases
- Ammonia lyases
- Hydroxynitrile lyases
- Carbonic anhydrases
- Carbon monoxide dehydrogenase
- Alkene monooxygenase, and
- Cysteine synthase.

Other enzymes may also be imagined.

In one or more embodiments, the micro-organisms or enzymes are dependent on oxygen.

In one or more embodiments, the oxygen dependent enzyme is selected from the group of oxidases, mono oxygenases, and dioxygenase.

In one or more embodiments, the oxygen dependent enzyme is glucose oxidase, or galactose oxidase.

As low dispersed flow is very dependent on the diffusivity of the solutes and the large size of enzymes translates into a two orders of magnitude lower diffusivity compared to small molecules (10^{-11} to 10^{-9} m^2/s). The axial dispersion of enzymes will therefore be much more pronounced, meaning they are more dispersed along the length of the channel compared to the small molecule reactants and the resulting products. It is therefore an advantage to make sure that the enzyme concentration in the entire reactor volume is constant.

This is obtained by achieving steady state with respect to the enzyme concentration and hereafter keeping the enzyme feed concentration constant, independent of the flowrate. It can therefore be assumed that the degree of dispersion will be solely dependent upon the diffusion coefficients of the substrate(s) and product(s).

In one or more embodiments, the method further comprises the step of obtaining the concentration of the enzyme or the micro-organism from the generated spectroscopic data in real time.

In one or more embodiments, the method further comprises the step of supplying fluids to the tube-in-tube reactor thereby creating a bubble-free aeration system.

In one or more embodiments, the method further comprises the step of supplying oxygen to the tube-in-tube reactor thereby creating a bubble-free aeration system.

The invention will in the following be described in further details in the connection with the drawings.

Brief description of the drawings

Figure 1 shows an example of a system for automated kinetic characterization in a tube-in-tube reactor.

Figure 2 shows a cut through view of the tube-in-tube.

Figure 3 shows the specific initial reaction rate vs oxygen concentration at different levels of glucose in Batch (x) and tube-in-tube reactor (TiTR) (o).

Figure 4 shows data collected at 1 bar and 6 bar plotted and fitted together.

Figure 5 shows the tube-in-tube reactor operating in steady state where sample points are collected after 4 residence times and then compared with experiments conducted with the ramp method.

Figure 6 shows the applied calibration curve.

Figure 7 shows experiments revealing that for all investigated enzyme concentrations the activity scaled linearly with enzyme concentration, which confirm that no mass-transfer limitations are experienced.

Figure 8 shows the residence time distribution experiments, step change response with a flow rate of 25 µL/min.

Description of preferred embodiments

Figure 1 shows an example of a system 100 for automated kinetic characterization in a tube-in-tube reactor 102. The system 100 comprises the tube-in-tube reactor 102 which comprises an outer tube 104 extending from an inlet end 106 to an outlet end 108. The outer tube 104 is adapted for containing one or more fluids such as e.g. gases, which have been humidified in a humidifier 109.

The outer tube may have an inner diameter of $d_{1,1}$ = 0.5-10.0 mm, such as 1.50-1.65 mm, e.g. 1.59 mm and an outer diameter of $d_{1,1}$ = 1.4-13.0 mm, such as 3.0-3.4 mm, e.g. 3.2 mm.

In figure 1, two boxes 110, 111 are shown in the first fluid delivery system. The boxes may be flow meters, or fluid delivery devices. The two boxes 110, 111 symbolize placeholders for either flowmeters or pumps. If the fluids are gasses, the two boxes 110, 111 will normally be gas cylinders. The first box 110 in figure 1 may contain oxygen whereas the second box 111

may contain nitrogen, which – together with the oxygen – is led through the humidifier 109 before being introduced into the outer tube 104.

The gasses are normally mixed before entering the humidifier 109. The gasses are humidified to ensure that there is no driving force for the water in the reaction mixture to diffuse from the inner tube to the outer tube.

The second fluid system includes two boxes 112,113 for delivering liquids. More boxes may also be imagined.

The fluid delivered to tube-in-tube reactor 102 is delivered to the outer 104 at the outside of the membrane constituting the inner tube 114, such that the fluid, e.g. oxygen can diffuse over the membrane and into the void inside the inner tube 114.

The system 100 shown in figure 1 further comprises a fluid delivering system 120, 121 – in figure 1 in the shape of a valve – for delivering the fluids to the outer tube 104 at or near the inlet end 106.

The tube-in-tube reactor 102 also comprises an inner tube 114 extending at least inside the outer tube 104 from an inlet end 116 to an outlet end 118. The inner tube 114 may be longer than the outer tube 104 at the inlet ends 106, 116 and/or at the outlet ends 108, 118.

The inner tube 114 is adapted for containing a liquid solution comprising one or more liquids. The liquids are delivered to the inner tube 114 at or near the inlet end 116 using one or more pumps 122, 124, 126 defining a flow rate of the one or more liquids in the inner tube 114. In figure 1, three syringe pumps 122, 124, 126 deliver the liquid solution to the inner tube 114, the latter being illustrated by the dotted line.

The three pumps 122, 124, 126 are drawing liquids from three reservoirs containing an enzyme/microorganism solution 142, a buffer solution 144 and an additional solvent 146. In one example these solutions 142, 144, 146 may be stock solutions of enzyme, buffer and substrate, respectively, such as e.g. a stock solutions of glucose oxidase, buffer and glucose, respectively. The three pumps 122, 124, 126 may be syringe pumps such as e.g. CAVRO XLP 60000 modular syringe pumps acquired from TECAN.

The outlets 123, 125, 127 of the three pumps 122, 124, 126 are mixed in a micro mixer 138, e.g. a 5 µl micro mixer from ASI, before entering the inner tube 104 of the tube-in-tube reactor 102.

At the outlet end 118 of the inner tube 114 is a spectrometric detector 200 for measuring spectra of the liquid solution having passed through the tube-in-tube reactor 102. The spectrometer 200 is positioned downstream of the tube-in-tube reactor 102.

The flow rate of the one or more liquids inside the inner tube 114 and the diameter $d_{2,1}$ of the inner tube (see figure 2) is adjusted to ensure a low dispersed flow of the one or more liquids with a Bodenstein number above 50. More specifically, the flow rate of the one or more liquids inside the inner tube provides a low dispersed flow of the one or more liquids with a Bodenstein number above 50. The flow rate is defined by specifics of the one or more pumps delivering the one or more liquids to the inner tube and the inner diameter of the inner tube. By “specifics” is meant that the liquid flow is not measured as such, but instead is ensured to be within a specific range by choosing a well calibrated, precise fluid delivery system, such as e.g. a syrringe pump or pressure driven flow.

The inner tube 114 is a semipermeable membrane made of a polymer material with high gas permeability, which ensures that gaseous substrates having passed through the humidifier 109 can be delivered from the outer tube 104 to the liquid reaction media inside the inner tube 114 while retaining the chemical resistance of traditional polymers. Mass flow controllers 128, 130 are used to vary the gas composition. The gas is wetted and possible also heated before entering the tube-in-tube reactor 102 in order to avoid the stripping of water through the inner tube 114.

The inner tube 114 may be made from Teflon AF-2400 and may be encased in a PTFE outer tube 104, the latter with low oxygen permeability. A mixture of oxygen and nitrogen is supplied in the space 115 between the two tubes, whereby the oxygen can be transferred to the liquid reaction mixture in the inner tube 114 through the membrane constituting the inner tube 114.

The small dimension of the inner tube maximizes the surface-to-volume ratio, which combined with the high oxygen permeability of Teflon AF-2400, enables very high supply rates. The inner tube 114 may have an inner diameter $d_{2,1}$ of 230µm and an outer diameter $d_{2,2}$ of 410 µm. This allows for operation that is very close to equilibrium between the reactor and gas phase despite a low driving force, i.e. the partial pressure of oxygen on both sides of

the membrane are very close to equilibrium. Additionally, by pressurizing both the inner and outer tube, the oxygen solubility in the reaction mixture can be increased proportionally.

Pressure regulators 132, 134 – possibly in combination with a manometer – are located at the outlet ends 108, 118 of both the two tubes 104, 114 in order to control and ensure an equal or higher pressure on the liquid side of the inner tube 114. By controlling the pressure and the gas composition, it is possible to control the oxygen concentration, since the oxygen transfer over the membrane is very fast.

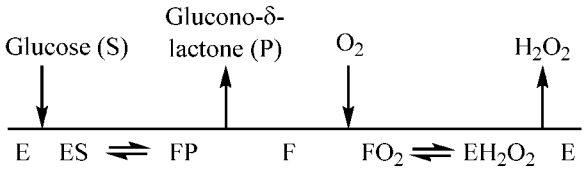
The length of the tube-in-tube reactor 102 shown in figure 1 is 3 m, but may vary within a range between 1-10 m.

The tube-in-tube reactor may be submerged in a water bath standing on a magnetic stirrer, maintained at 25°C by use of a temperature controller.

In figure 1, the liquid that exits the inner tube of the tube-in-tube reactor 102 enters an injection valve 140. The injection valve is also coupled to a mobile phase 141. When 100 nL of the liquid from the tube-in-tube reactor 102 is captured, the valve 140 is turned, and buffer is pumped through to push the liquid into the detector 200. The detector 200 may be a diode array detector, which allows for automated and continuous analysis of the composition of the outgoing liquid. The detector 200 measures the absorbance at wavelengths of 210-600 nm. The absorbance is measured simultaneously at each wavelength at a frequency of 20 Hz. The buffer may be supplied by a piston pump. Other types of spectrometers may also be imagined.

Example 1

In order to demonstrate the performance of the system, the well-known enzyme, glucose oxidase (GOx, E.C. 1.1.3.4), is selected. The GOx enzyme catalyzes the oxidation of glucose to glucono-δ-lactone, with molecular oxygen, which is reduced to hydrogen peroxide. Glucono-δ-lactone is then hydrolysed to gluconic acid and the formation can be followed spectrophotometrically. The formed hydrogen peroxide is removed by the addition of catalase, which facilitates its instantaneous conversion into water and half the stoichiometric amount of oxygen. The removal of hydrogen peroxide forces the reaction to proceed in a unidirectional manner and protects GOx from oxidation. GOx has been shown to follow a ping-pong bi bi reaction mechanism as illustrated in Scheme 1 and the reaction rate can consequently be derived using Eq. 8. In scheme 1, E denotes the oxidized free form of the enzyme whereas F denotes the reduced form of the free enzyme.



Scheme 1

$$\frac{r}{[E]} = \frac{k_{cat} S\, O}{S\, O + K_{mO}\, S + K_{mS}\, O}$$

Eq. 8

The flow manipulation method applied to produce batch data from the setup, requires an accurate determination of the reactor volume. Here residence time distribution experiments were used to determine the volume of the tube-in-tube reactor to be 155 ± 1.8 µL (see figure 8).

The results of the flow method were compared with steady-state operation, where it was shown that the setup indeed produces time-series data even with the addition of a slow diffusing catalyst. Finally, in order to validate the enzyme kinetics measured in the tube-in-tube reactor, experiments using an aerated stirred tank reactor with adjustable oxygen/nitrogen feed were conducted. The comparison reveals a very good correlation between the systems and the combined result of the validation experiments illustrates that confidence can be placed on the kinetics determined in the tube-in-tube reactor setup as shown in figure 3.

Figure 3 shows the specific initial reaction rate vs oxygen concentration at different levels of glucose in Batch (marked with x) and tube-in-tube reactor (marked with o). The concentration of glucose is 400 mM in first curve 301 from the top, 200 mM in the second curve 302 from the top, 100 mM in the third curve 303 from the top, and 25 mM in the fourth curve 304 form the top. The full lines are the model fit to the tube-in-tube reactor results using Eq. 8.

The experiments were carried out with 100 mM potassium phosphate buffer at pH 7 and Glucose Oxidase 0.1 mg/mL at 25 °C and at atmospheric pressure. The initial rate data collected for the batch system is here scaled by a factor 0.79, this has been done to correct for time dependent degradation of the enzyme formulation between conducting the batch experiments and the TiTR experiments.

The fit of Eq. 8 to the data revealed a relatively high Michaelis constant for oxygen of 0.52 mM (see Table 1), which also is seen from the unsaturated enzyme kinetics observed at high glucose concentrations and atmospheric pressure shown in figure 3. In the scientific literature, it has frequently been proposed that in order to reliably quantify Michaelis constants it is necessary to measure kinetics at a sufficient range of substrate concentrations at best 5-fold and preferably 10-fold the true K_M, in both directions. In this setup, this was achieved by increasing the operating pressure of the setup to 6 bar to increase the solubility of oxygen to 7.13 mM (using pure O_2 at 25 °C). Enzyme saturation was thereby obtained even at the highest concentration of glucose as shown in figure 4, enabling a more reliable prediction of all the kinetic parameters (see Table 1).

Figure 4 shows data collected at 1 atm (0.14-1.3 mM O_2) and 5 barg (0.9-7.13 mM O_2) plotted and fitted together. The concentration of glucose is 400 mM in the first curve 401 from the top, 200 mM in the second curve 402 from the top, 100 mM in the third curve 403 from the top, and 25 mM in the fourth curve 404 form the top. The 'o' marks the tube-in-tube reactor results, and the full lines are the model fit. The experiments were carried out with 100 mM potassium phosphate buffer at pH 7 and Glucose Oxidase 0.1 mg/mL at 25 °C..

Table 1. Parameter estimations based on different experimental data, pressure is given as absolute pressure.

Parameter	Batch reactor (1 bar)	Tube-in-tube reactor (1 bar)	Tube-in-tube reactor (1 bar + 6 bar)
k_{cat} [µmol min^{-1} mg^{-1}]*	17.58 ± 0.62**	17.78 ± 1.39	17.82 ± 0.47
K_{MO} [mM]	0.45 ± 0.04	0.51 ± 0.09	0.52 ± 0.03
K_{MS} [mM]	73.1 ± 6.87	75.2 ± 9.38	74.57 ± 5.55

* Based on milligrams of liquid formulation

** The batch data is scaled by a factor 0.79 to correct for time dependent degradation of the enzyme formulation between the experiments

The small dimension of the system according to the invention makes it possible to collect one initial rate measurement per 1.4 mL, which is much less than the 150 mL used in the alternative sparged batch setup.

In general, when running the experiments on enzymes using oxygen and nitrogen as the fluids, the pumps are initially primed with the designated substrates and enzyme solutions, and are hereafter set to run continuously to reach steady state. In parallel, the mass flow controllers are set to produce a defined mixture of oxygen and nitrogen. To ensure steady

state, the system is set to wait a total of 3 residence times of 2 min. and a sample is hereafter collected along with starting the ramp method. The method follows the procedure published by Moore and Jensen with an alpha of 0.5, which determines the rate at which residence time changes with real time and each flow ramp was set to run 15 minutes real time.

The outlet of the inner tube in the tube-in-tube reactor is connected to an injection valve with two 5 µL sample loops and samples were injected every minute by switching positions on the valve. The valve acts as a connector of two isolated systems, where one sample loop is flushed by the mobile phase with the piston pump and carried to the detector while the other is being filled by the outlet of the reactor. A switch in position of the injection valve is therefore connecting the full sample loop with the mobile phase and the sample is carried to the detector. Detection was operated as follows: the detector is balanced, and once it is ready, the valve is set to switch position, spectral data of 210-600 nm is hereafter collected over time with a frequency of 20 Hz. The recorded full spectra pulse is hereafter used in data analysis, however for this specific purpose, only the signal collected at 210 nm was used.

Design of experiments was carried out in a excel spreadsheet, where flow rate for each syringe pumps and the mass flow controllers are defined. The values were hereafter loaded into LabVIEW, which then processes the setpoints sequentially. Other software programs may naturally be used instead of LabVIEW.

In order to validate the flow ramp method developed by Moore and Jensen for enzyme catalysed reactions it is necessary to see if low dispersed flow is produced at the investigated conditions. This can be done by collecting steady state sample points after 4 residence times and compared them with ramp experiments. The results are shown in figure 5 and strongly indicate that there is little to no difference between the two sampling methods why it can be concluded that low dispersed flow conditions are met using the system and the method of the invention.

The experiments shown in figure 5 is carried out with 0.1 mg/mL GOx enzyme, 100 mM glucose, 0.52 mM O_2 and with 100 mM Potassium phosphate buffer at pH 7.

The production of gluconic acid can be followed by integrating the retention time peak at 210 nm. Figure 6 shows the applied calibration curve.

In order to confirm that the reaction was not limited by the mass-transfer of oxygen across the inner tube, it is necessary to observe a linear relationship between the enzyme concentration and activity. As shown in figure 7, experiments reveal that for all investigated enzyme concentrations the activity scaled linearly with the enzyme concentration, thereby confirming that no mass transfer limitations occurred

For the ramp method to be correct it is necessary to have a precise determination of the reactor volume. Here residence time distribution experiments can be used to determine the volume. Figure 8 shown the residence time distribution experiments, step change response with a flow rate of 25 µL/min. The volume is determined to be 155 ± 1.8 µL by the method described in the textbook by Fogler.

Items

1. System for automated kinetic characterization in a tube-in-tube reactor, the system comprising:
   - at least one tube-in-tube reactor extending along a length from an inlet to an outlet end, the at least one tube-in-tube reactor comprising:
     - an outer tube extending from an inlet end to an outlet end, wherein the outer tube is adapted for containing one or more fluids, and
     - an inner tube extending at least inside the outer tube from an inlet end to an outlet end, the inner tube having an inner diameter, wherein the inner tube is adapted for containing a liquid solution comprising one or more liquids;
   - one or more pumps for delivering the one or more liquids to the inner tube at or near the inlet end of the inner tube, wherein the one or more pumps defines a flow rate of the one or more liquids in the inner tube;
   - one or more fluid delivering system for delivering the one or more fluids to the outer tube at or near the inlet end of the outer tube;
   - a spectrometric detector for measuring spectra of the liquid solution having passed through the tube-in-tube reactor, the spectrometer being positioned downstream of the tube-in-tube reactor;
   - an outer tube pressure regulator at the outlet end of the outer tube, and
   - an inner tube pressure regulator at the outlet end of the inner tube,

   wherein the one or more fluids may diffuse from the outer tube and into the inner tube and wherein the one or more fluids are present in the inner tube and the outer tube at

a saturation level, which is the same along a majority of the length of the tube-in-tube reactor;

wherein the pressure regulators control and ensure an equal or higher pressure inside the inner tube than inside the outer tube,

wherein the flow rate of the one or more liquids inside the inner tube provides a low dispersed flow of the one or more liquids with a Bodenstein number above 50, and wherein the flow rate is defined by specifics of the one or more pumps delivering the one or more liquids to the inner tube and the inner diameter of the inner tube, and

wherein the inner tube is non-porous or porous where all pores is having pore sizes below 300 nm in diameter.

2. System according to item 1, wherein the outer and/or the inner pressure regulators are selected from the group of:
   - a seat,
   - a needle,
   - a relief
   - a control valve,
   - a piece of tubing with a significant pressure drop at the flow rate,
   - a water column.

3. System according to any preceding item, wherein the inner pressure regulator and/or the outer pressure regulator is controlled by means of a computer or a digital circuit.

4. System according to any preceding item, wherein the inner pressure regulator and/or the outer pressure regulator is actuated so that the pressure of the inner tube and/or the outer tube, respectively, is adjustable to such that different pressure set points is obtainable during operation.

5. System according to any preceding item, wherein the system comprises one tube-in-tube reactor.

6. System according to any of the items 1-4, wherein the system comprises a plurality of tube-in-tube reactors arranged in serial.

7. System according to any of the items 1-4, wherein the system comprises a plurality of tube-in-tube reactors arranged in parallel.

8. System according to item 6 or 7, wherein the multiple of tube-in-tube reactors includes two tube-in-tube reactors, or three tube-in-tube reactors, or four tube-in-tube reactors, or five tube-in-tube reactors.

9. System according to any preceding item, wherein the Bodenstein number is above 100, or above 200, or above 300, or above 400, or above 500, or above 600.

10. System according to any preceding item, wherein the system further comprises an outer tube pressure monitor at the outlet end of the outer tube, and an inner tube pressure monitor at the outlet end of the inner tube, the pressure monitors e.g. being a manometer or a pressure transducer.

11. System according to any preceding item, wherein the one or more pumps includes a first pump for delivering a liquid solution comprising a first enzyme or micro-organism type to the inner tube.

12. System according to any preceding item, wherein the one or more pumps includes a second pump for delivering a substrate solution to the inner tube.

13. System according to any preceding item, wherein the one or more pumps includes a third pump for delivering a buffer solution to the inner tube.

14. System according to any preceding item, wherein the one or more pumps are single pumps for delivering the one or more liquids to the inner tube in controlled portions.

15. System according to any preceding item, wherein the one or more pumps are syringe pumps.

16. System according to any preceding item, wherein the inner tube is made from an inner tube material having a high oxygen permeability and the outer tube is made from an outer tube material having a low oxygen permeability being significantly lower than the oxygen permeability of the inner tube material.

17. System according to item 16, wherein the permeability of the outer tube material is at least 100 times lower than that of the inner tube.

18. System according to any preceding item, wherein the inner tube material is selected from the group of:
    - Fluorinated polymers, such as amorphous copolymer of tetrafluroethylene (TFE), polytetrafluoroethylene, and 2,2-bis-triflouromethyl-4,5-diflouro-1,3dioxole (PDD);
    - Perfluorosulfonate polymers;
    - Silicone based polymers, such as polydimethylsiloxane (PDMS) and poly(1-trimethylsilyl-1-propyne) (PTMSP);
    - Polyethylene (PE);
    - Polypropylene (PP), and
    - Polycarbonate, and

19. System according to item 18, wherein the inner tube material is a polytetrafluoroethylene, preferably an amorphous copolymer of TFE and PDD.

20. System according to any preceding item, wherein the outer tube material is selected form the group of:
    - Polytetrafluoroethylene (PTFE);
    - Polyethylene terephthalate (PET);
    - Polyvinylidene fluoride (PVDF);
    - Ethylene ChloroTriFluoroEthylene (ECTFE);
    - PolyEther Ether Ketone (PEEK);
    - Steel including stainless steel and steel alloys.

21. System according to item 20, wherein the outer tube material is polytetrafluoroethylene, preferably a semi-crystalline polytetrafluoroethylene.

22. System according to any preceding item, wherein the inner tube in the tube-in-tube reactor has a significantly higher oxygen permeability than the outer tube.

23. System according to any preceding item, wherein the one or more fluid delivering system for delivering the one or more fluids to the inner tube are mass flow apparatus or pumps, such as e.g. syringe pumps.

24. System according to any preceding item, wherein the saturation level of the one or more fluids is lower at the inlet end than at the outlet end of the inner tube.

25. System according to item 24, wherein the saturation level of the one or more fluid at the inlet end of the inner tube is between 0-21% and at the outlet end of the inner tube is between 50-100%, such as 80-100%, or 90-100%, or 95-100%, or 99-100%.

26. System according to any preceding item, wherein the one or more fluids delivered to the outer tube are mixed in their full range before entering the outer tube.

27. System according to any preceding items, wherein the one or more fluids are gases.

28. System according to item 27, wherein the system further comprises a humidifier for humidifying the one or more gases before the one or more gases enters the outer tube, and wherein the system further comprises one or more gas mass flow controllers for controlling the gas flow into the humidifier.

29. System according to item 28, wherein the humidifier is adapted for introducing concentrations up to 100% humidity at all temperatures within the tube-in-tube reactor.

30. System according to any preceding items, wherein the system further comprises one or more gas mass flow controllers for controlling the gas flow into the humidifier.

31. System according to any preceding items, wherein the one or more gases is selected from the list of:

- Oxygen (O_2),
- Nitrogen (N_2),
- Ammonia (NH_3),
- Carbon monoxide (CO),
- Carbon dioxide (CO_2),
- Hydrogen (H_2),
- Methane (CH_3),
- Ozone (O_3),
- Ethane (C_2H_6),
- Ethylene (C_2H_4),
- Propane (C_3H_8),
- Propene (C_3H_6),
- Butane (C_4H_{10}),
- Butylene (C_4H_8),
- Hydrogen sulphide (H_2S),

- Nitrogen oxides (NO and/or NO_2),
- Sulfur dioxide (SO_2),
- Hydrogen cyanide (HCN).

32. System according to any preceding items, wherein the one of the one or more gases includes N_2 contained in a nitrogen receptacle.

33. System according to any preceding items, wherein the one of the one or more gases includes O_2 contained in an oxygen receptacle.

34. System according to items 33, wherein one or more mass flow controllers varies the oxygen amount in the range 0-100% O_2.

35. System according to any of the items 1-26, wherein the one or more fluids are liquids.

36. System according to any preceding item, wherein the tube-in-tube reactor has a length which is adjusted to give a steady flow for the one or more pumps.

37. System according to any preceding item, wherein the tube-in-tube reactor has a length between 1-10 meter, or 1-7 meter, or 1-5 meter, or 2-4 meter, or approximately 3 meter.

38. System according to any preceding item, wherein the inner tube has an inner diameter defining the opening for the liquid, the inner diameter of the inner tube being between 0.025-0.50 mm, or between 0.10-0.25 mm, or between 0.15-0.25 mm, or between 0.18-0.22 mm, or approximately 0.20 mm.

39. System according to item 38, wherein the inner tube has an outer diameter being twice as large as the inner diameter of the inner tube.

40. System according to any preceding item, wherein the inner tube has an outer diameter between 0.1-0.6 mm, or between 0.3-0.5 mm, or between 0.35-0.45 mm, or between 0.38-0.42 mm, or approximately 0.4 mm.

41. System according to any preceding item, wherein the inner tube has a wall thickness between 10-500 µm, such as 50-200 µm, or such as 50-100 µm.

42. System according to any preceding item, wherein the outer tube has an inner diameter defining the opening for the one or more fluids and the inner tube, the inner diameter of the outer tube being between 0.5-10.0 mm, or between 0.5-7.5 mm, or between 0.5-5.0 mm, or between 0.5-3.2 mm, or between 1.0-2.0 mm, or between 1.2-1.8 mm, or between 1.5-1.7 mm, or approximately 1.6 mm.

43. System according to any preceding item, wherein the outer tube has an outer diameter between 1.4-13.0 mm, or between 1.5-10.0 mm, or between 1.58-6.4 mm, or between 2.8-4.0 mm, or between 3.0-3.6 mm, or between 3.1-3.3 mm, or approximately 3.2 mm.

44. System according to any preceding item further comprising a heating/cooling device for controlling the temperature of the fluids before entering the tube-in-tube reactor.

45. System according to any preceding item, wherein the outlet end of the inner tube is connected to the spectrometric detector by means of an injection valve.

46. System according to item 45, wherein the injection valve is a switch valve.

47. System according to item 45 or 46, and wherein the injection valve is controlled manually or electronically.

48. System according to any of the items 1-45, wherein the spectrometric detector is coupled to the inner tube in an in-line manner.

49. System according to any preceding item, wherein the spectrometric detector is selected from the group of:

- ultra violet-visible detector or spectrometer;
- mass spectrometers;
- Raman spectrometers;
- NMR spectrometers;
- florescence detectors or spectrometers, and
- infrared spectrometers.

50. System according to any preceding item, wherein the spectrometric detector is an ultra violet-visible detector or spectrometer.

51. System according to any preceding item, wherein the tube-in-tube reactor further comprises one or more additional inner tubes arranged in parallel inside the outer tube.

52. System according to item 51, wherein each of the multiple inner tubes inside the outer tube is connected to a spectrometric detector.

53. Use of a tube-in-tube reactor according to any of the items 1-52 for collecting kinetic data of reactions catalysed by micro-organisms or enzymes, the reactions including:
- Oxidation reactions catalysed by enzymes such as oxidases, dehydrogenases
- Hydrogenation reactions catalysed by enzymes such as hydrogenases;
- Amination reactions catalysed by enzymes such as transaminases; and
- Carboxylation reactions catalysed by enzymes such as carboxylases.

54. Method for collecting kinetic data of reactions catalysed by micro-organisms or enzymes, the method comprising the steps of:
- providing a tube-in-tube reactor according to any of the items 1-52;
- operating the tube-in-tube reactor in a low dispersed flow regime
- supplying the tube-in-tube reactor with an enzyme solution or a micro-organism suspension; and
- generating time-series of spectroscopic data.

55. Method according to item 54, wherein the micro-organisms or enzymes are gas dependent.

56. Method according to item 55, wherein the micro-organisms or enzymes are selected from the group of:
- Hydrogenases
- Ammonia lyases
- Hydroxynitrile lyases
- Carbonic anhydrases
- Carbon monoxide dehydrogenase
- Alkene monooxygenase, and
- Cysteine synthase.

57. Method according to item 55, wherein the micro-organisms or enzymes are dependent on oxygen.

58. Method according to item 57, wherein the oxygen dependent enzyme is selected from the group of oxidases, mono oxygenases, and dioxygenase.

59. Method according to item 58, wherein the oxygen dependent enzyme is glucose oxidase, or galactose oxidase.

60. Method according to any of the items 54-59 further comprising the step of obtaining the concentration of the enzyme or the micro-organism from the generated spectroscopic data in real time.

61. Method according to any of the items 54-60 supplying fluids to the tube-in-tube reactor thereby creating a bubble-free aeration system.

62. Method according to any of the items 54-61 further comprising the step of supplying oxygen to the tube-in-tube reactor thereby creating a bubble-free aeration system.

References

100	system for automated kinetic characterization in a tube-in-tube reactor
102	tube-in-tube reactor
104	outer tube
106	inlet end of the outer tube
108	outlet end of the outer tube
109	humidifier
110	first box in the first fluid delivery system
111	second box in the first fluid delivery system
112	first box in the second fluid delivery system
113	second box in the second fluid delivery system
114	inner tube
115	space between the inner tube and the outer tube
116	inlet end of the inner tube
118	outlet end of the inner tube
120	fluid delivering system
121	fluid delivering system
122	pump, e.g. a syringe pump
123	outlet of the pump 122
124	pump, e.g. a syringe pump
125	outlet of the pump 124
126	pump, e.g. a syringe pump
127	outlet of the pump 126
128	mass flow controller
130	mass flow controller
132	pressure regulator at the outlet end of the outer tube
134	pressure regulator at the outlet end of the inner tube
136	outlet of the first pump
138	micro mixer
140	injection valve
141	mobile phase
142	enzyme solution or microorganism solution
144	buffer solution
146	solvent
200	spectrometer

$d_{1,1}$ inner diameter of the outer tube 104

$d_{1,2}$ outer diameter of the outer tube 104

$d_{2,1}$ inner diameter of the inner tube 114

$d_{2,2}$ outer diameter of the inner tube 114

5

Claims

1. System for automated kinetic characterization in a tube-in-tube reactor, the system comprising:
   - at least one tube-in-tube reactor extending along a length from an inlet to an outlet end, the at least one tube-in-tube reactor comprising:
     - an outer tube extending from an inlet end to an outlet end, wherein the outer tube is adapted for containing one or more fluids, and
     - an inner tube extending at least inside the outer tube from an inlet end to an outlet end, the inner tube having an inner diameter, wherein the inner tube is adapted for containing a liquid solution comprising one or more liquids;
   - one or more pumps for delivering the one or more liquids to the inner tube at or near the inlet end of the inner tube, wherein the one or more pumps defines a flow rate of the one or more liquids in the inner tube;
   - one or more fluid delivering system for delivering the one or more fluids to the outer tube at or near the inlet end of the outer tube;
   - a spectrometric detector for measuring spectra of the liquid solution having passed through the tube-in-tube reactor, the spectrometer being positioned downstream of the tube-in-tube reactor;
   - an outer tube pressure regulator at the outlet end of the outer tube, and
   - an inner tube pressure regulator at the outlet end of the inner tube,

   wherein the one or more fluids may diffuse from the outer tube and into the inner tube and wherein the one or more fluids are present in the inner tube and the outer tube at a saturation level, which is the same along a majority of the length of the tube-in-tube reactor;

   wherein the pressure regulators control and ensure an equal or higher pressure inside the inner tube than inside the outer tube,

   wherein the flow rate of the one or more liquids inside the inner tube provides a low dispersed flow of the one or more liquids with a Bodenstein number above 50, and wherein the flow rate is defined by specifics of the one or more pumps delivering the one or more liquids to the inner tube and the inner diameter of the inner tube, and

   wherein the inner tube is non-porous or porous where all pores is having pore sizes below 300 nm in diameter.

2. System according to claim 1, wherein the outer and/or the inner pressure regulators are selected from the group of:
   - a seat,
   - a needle,
   - a relief
   - a control valve,
   - a piece of tubing with a significant pressure drop at the flow rate,
   - a water column.

3. System according to any preceding claim, wherein the inner pressure regulator and/or the outer pressure regulator is controlled by means of a computer or a digital circuit.

4. System according to any preceding claim, wherein the inner pressure regulator and/or the outer pressure regulator is actuated so that the pressure of the inner tube and/or the outer tube, respectively, is adjustable to such that different pressure set points is obtainable during operation.

5. System according to any preceding claim, wherein the system comprises one tube-in-tube reactor.

6. System according to any of the claims 1-4, wherein the system comprises a plurality of tube-in-tube reactors arranged in serial.

7. System according to any of the claims 1-4, wherein the system comprises a plurality of tube-in-tube reactors arranged in parallel.

8. System according to claim 6 or 7, wherein the multiple of tube-in-tube reactors includes two tube-in-tube reactors, or three tube-in-tube reactors, or four tube-in-tube reactors, or five tube-in-tube reactors.

9. System according to any preceding claim, wherein the Bodenstein number is above 100, or above 200, or above 300, or above 400, or above 500, or above 600.

10. System according to any preceding claim, wherein the system further comprises an outer tube pressure monitor at the outlet end of the outer tube, and an inner tube pressure monitor at the outlet end of the inner tube, the pressure monitors e.g. being a manometer or a pressure transducer.

11. System according to any preceding claim, wherein the one or more pumps includes a first pump for delivering a liquid solution comprising a first enzyme or micro-organism type to the inner tube.

12. System according to any preceding claim, wherein the one or more pumps includes a second pump for delivering a substrate solution to the inner tube.

13. System according to any preceding claim, wherein the one or more pumps includes a third pump for delivering a buffer solution to the inner tube.

14. System according to any preceding claim, wherein the one or more pumps are single pumps for delivering the one or more liquids to the inner tube in controlled portions.

15. System according to any preceding claim, wherein the one or more pumps are syringe pumps.

16. System according to any preceding claim, wherein the inner tube is made from an inner tube material having a high oxygen permeability and the outer tube is made from an outer tube material having a low oxygen permeability being significantly lower than the oxygen permeability of the inner tube material.

17. System according to claim 16, wherein the permeability of the outer tube material is at least 100 times lower than that of the inner tube.

18. System according to any preceding claim, wherein the inner tube material is selected from the group of:

- Fluorinated polymers, such as amorphous copolymer of tetrafluroethylene (TFE), polytetrafluoroethylene, and 2,2-bis-triflouromethyl-4,5-diflouro-1,3dioxole (PDD);
- Perfluorosulfonate polymers;
- Silicone based polymers, such as polydimethylsiloxane (PDMS) and poly(1-trimethylsilyl-1-propyne) (PTMSP);
- Polyethylene (PE);
- Polypropylene (PP), and
- Polycarbonate, and

19. System according to claim 18, wherein the inner tube material is a polytetrafluoroethylene, preferably an amorphous copolymer of TFE and PDD.

20. System according to any preceding claim, wherein the outer tube material is selected form the group of:
    - Polytetrafluoroethylene (PTFE);
    - Polyethylene terephthalate (PET);
    - Polyvinylidene fluoride (PVDF);
    - Ethylene ChloroTriFluoroEthylene (ECTFE);
    - PolyEther Ether Ketone (PEEK);
    - Steel including stainless steel and steel alloys.

21. System according to claim 20, wherein the outer tube material is polytetrafluoroethylene, preferably a semi-crystalline polytetrafluoroethylene.

22. System according to any preceding claim, wherein the inner tube in the tube-in-tube reactor has a significantly higher oxygen permeability than the outer tube.

23. System according to any preceding claim, wherein the one or more fluid delivering system for delivering the one or more fluids to the inner tube are mass flow apparatus or pumps, such as e.g. syringe pumps.

24. System according to any preceding claim, wherein the saturation level of the one or more fluids is lower at the inlet end than at the outlet end of the inner tube.

25. System according to claim 24, wherein the saturation level of the one or more fluid at the inlet end of the inner tube is between 0-21% and at the outlet end of the inner tube is between 50-100%, such as 80-100%, or 90-100%, or 95-100%, or 99-100%.

26. System according to any preceding claim, wherein the one or more fluids delivered to the outer tube are mixed in their full range before entering the outer tube.

27. System according to any preceding claim, wherein the one or more fluids are gases.

28. System according to claim 27, wherein the system further comprises a humidifier for humidifying the one or more gases before the one or more gases enters the outer

tube, and wherein the system further comprises one or more gas mass flow controllers for controlling the gas flow into the humidifier.

29. System according to claim 28, wherein the humidifier is adapted for introducing concentrations up to 100% humidity at all temperatures within the tube-in-tube reactor.

30. System according to any preceding claim, wherein the system further comprises one or more gas mass flow controllers for controlling the gas flow into the humidifier.

31. System according to any preceding claim, wherein the one or more gases is selected from the list of:

- Oxygen (O_2),
- Nitrogen (N_2),
- Ammonia (NH_3),
- Carbon monoxide (CO),
- Carbon dioxide (CO_2),
- Hydrogen (H_2),
- Methane (CH_3),
- Ozone (O_3),
- Ethane (C_2H_6),
- Ethylene (C_2H_4),
- Propane (C_3H_8),
- Propene (C_3H_6),
- Butane (C_4H_{10}),
- Butylene (C_4H_8),
- Hydrogen sulphide (H_2S),
- Nitrogen oxides (NO and/or NO_2),
- Sulfur dioxide (SO_2),
- Hydrogen cyanide (HCN).

32. System according to any preceding claim, wherein the one of the one or more gases includes N_2 contained in a nitrogen receptacle.

33. System according to any preceding claim, wherein the one of the one or more gases includes O_2 contained in an oxygen receptacle.

34. System according to claims 33, wherein one or more mass flow controllers varies the oxygen amount in the range 0-100% O_2.

35. System according to any of the claims 1-26, wherein the one or more fluids are liquids.

36. System according to any preceding claim, wherein the tube-in-tube reactor has a length which is adjusted to give a steady flow for the one or more pumps.

37. System according to any preceding claim, wherein the tube-in-tube reactor has a length between 1-10 meter, or 1-7 meter, or 1-5 meter, or 2-4 meter, or approximately 3 meter.

38. System according to any preceding claim, wherein the inner tube has an inner diameter defining the opening for the liquid, the inner diameter of the inner tube being between 0.025-0.50 mm, or between 0.10-0.25 mm, or between 0.15-0.25 mm, or between 0.18-0.22 mm, or approximately 0.20 mm.

39. System according to claim 38, wherein the inner tube has an outer diameter being twice as large as the inner diameter of the inner tube.

40. System according to any preceding claim, wherein the inner tube has an outer diameter between 0.1-0.6 mm, or between 0.3-0.5 mm, or between 0.35-0.45 mm, or between 0.38-0.42 mm, or approximately 0.4 mm.

41. System according to any preceding claim, wherein the inner tube has a wall thickness between 10-500 µm, such as 50-200 µm, or such as 50-100 µm.

42. System according to any preceding claim, wherein the outer tube has an inner diameter defining the opening for the one or more fluids and the inner tube, the inner diameter of the outer tube being between 0.5-10.0 mm, or between 0.5-7.5 mm, or between 0.5-5.0 mm, or between 0.5-3.2 mm, or between 1.0-2.0 mm, or between 1.2-1.8 mm, or between 1.5-1.7 mm, or approximately 1.6 mm.

43. System according to any preceding claim, wherein the outer tube has an outer diameter between 1.4-13.0 mm, or between 1.5-10.0 mm, or between 1.58-6.4 mm, or between 2.8-4.0 mm, or between 3.0-3.6 mm, or between 3.1-3.3 mm, or approximately 3.2 mm.

44. System according to any preceding claim further comprising a heating/cooling device for controlling the temperature of the fluids before entering the tube-in-tube reactor.

45. System according to any preceding claim, wherein the outlet end of the inner tube is connected to the spectrometric detector by means of an injection valve.

46. System according to claim 45, wherein the injection valve is a switch valve.

47. System according to claim 45 or 46, and wherein the injection valve is controlled manually or electronically.

48. System according to any of the claims 1-45, wherein the spectrometric detector is coupled to the inner tube in an in-line manner.

49. System according to any preceding claim, wherein the spectrometric detector is selected from the group of:

- ultra violet-visible detector or spectrometer;
- mass spectrometers;
- Raman spectrometers;
- NMR spectrometers;
- florescence detectors or spectrometers, and
- infrared spectrometers.

50. System according to any preceding claim, wherein the spectrometric detector is an ultra violet-visible detector or spectrometer.

51. System according to any preceding claim, wherein the tube-in-tube reactor further comprises one or more additional inner tubes arranged in parallel inside the outer tube.

52. System according to claim 51, wherein each of the multiple inner tubes inside the outer tube is connected to a spectrometric detector.

53. Use of a tube-in-tube reactor according to any of the claims 1-52 for collecting kinetic data of reactions catalysed by micro-organisms or enzymes, the reactions including:

- Oxidation reactions catalysed by enzymes such as oxidases, dehydrogenases

- Hydrogenation reactions catalysed by enzymes such as hydrogenases;
- Amination reactions catalysed by enzymes such as transaminases; and
- Carboxylation reactions catalysed by enzymes such as carboxylases.

54. Method for collecting kinetic data of reactions catalysed by micro-organisms or enzymes, the method comprising the steps of:
    - providing a tube-in-tube reactor according to any of the claims 1-52;
    - operating the tube-in-tube reactor in a low dispersed flow regime
    - supplying the tube-in-tube reactor with an enzyme solution or a micro-organism suspension; and
    - generating time-series of spectroscopic data.

55. Method according to claim 54, wherein the micro-organisms or enzymes are gas dependent.

56. Method according to claim 55, wherein the micro-organisms or enzymes are selected from the group of:
    - Hydrogenases
    - Ammonia lyases
    - Hydroxynitrile lyases
    - Carbonic anhydrases
    - Carbon monoxide dehydrogenase
    - Alkene monooxygenase, and
    - Cysteine synthase.

57. Method according to claim 55, wherein the micro-organisms or enzymes are dependent on oxygen.

58. Method according to claim 57, wherein the oxygen dependent enzyme is selected from the group of oxidases, mono oxygenases, and dioxygenase.

59. Method according to claim 58, wherein the oxygen dependent enzyme is glucose oxidase, or galactose oxidase.

60. Method according to any of the claims 54-59 further comprising the step of obtaining the concentration of the enzyme or the micro-organism from the generated spectroscopic data in real time.

61. Method according to any of the claims 54-60 supplying fluids to the tube-in-tube reactor thereby creating a bubble-free aeration system.

62. Method according to any of the claims 54-61 further comprising the step of supplying oxygen to the tube-in-tube reactor thereby creating a bubble-free aeration system.

1/5

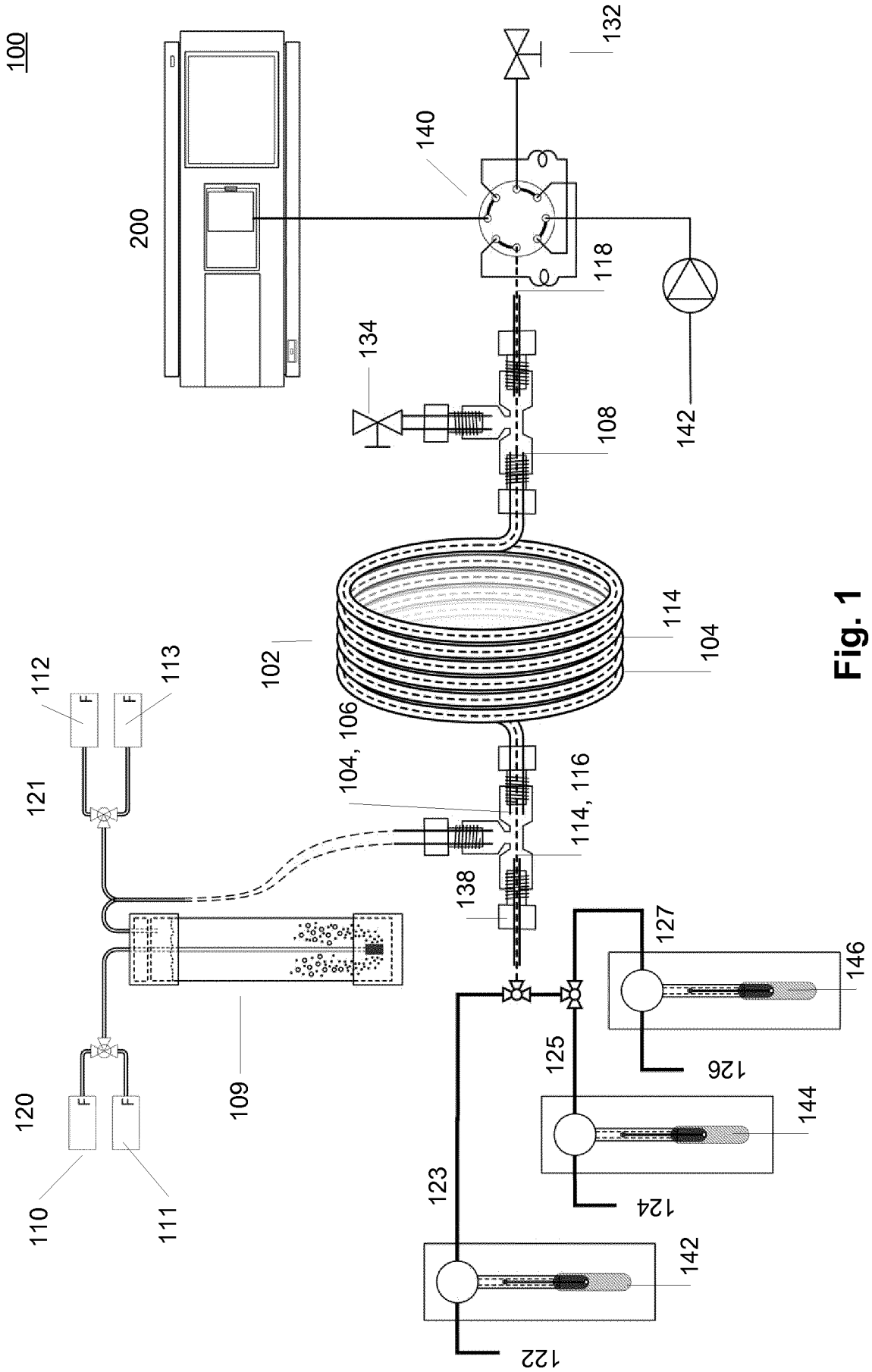

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Fig. 1

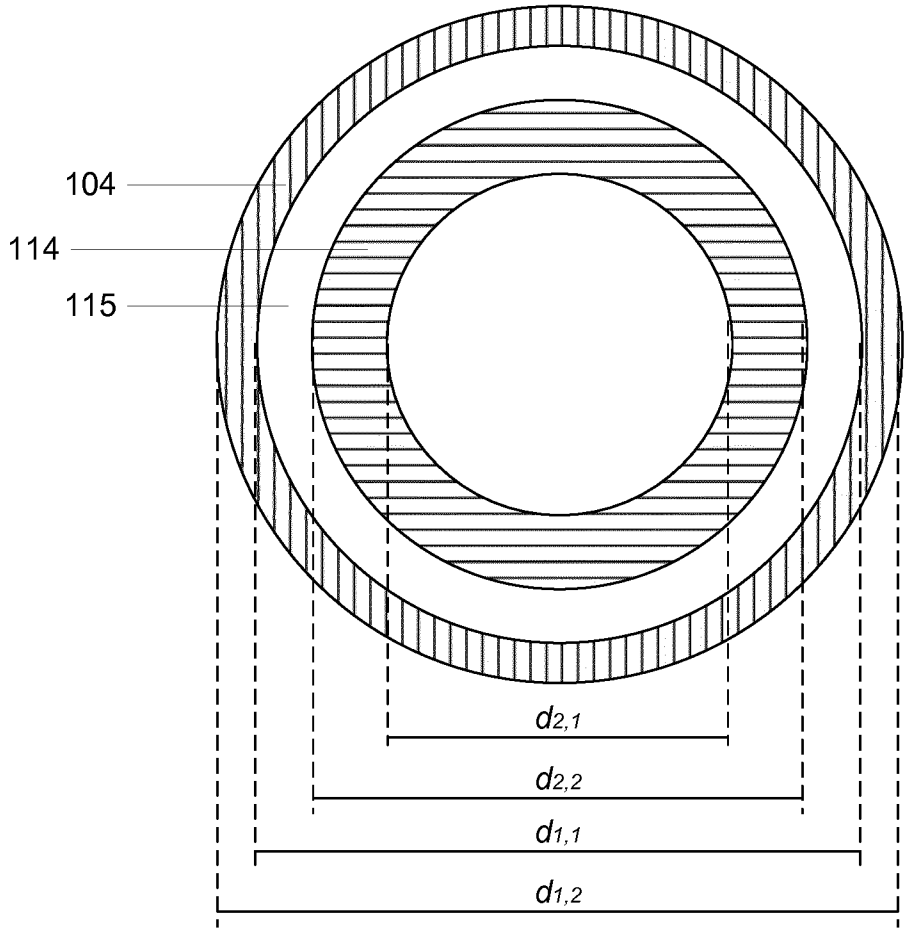
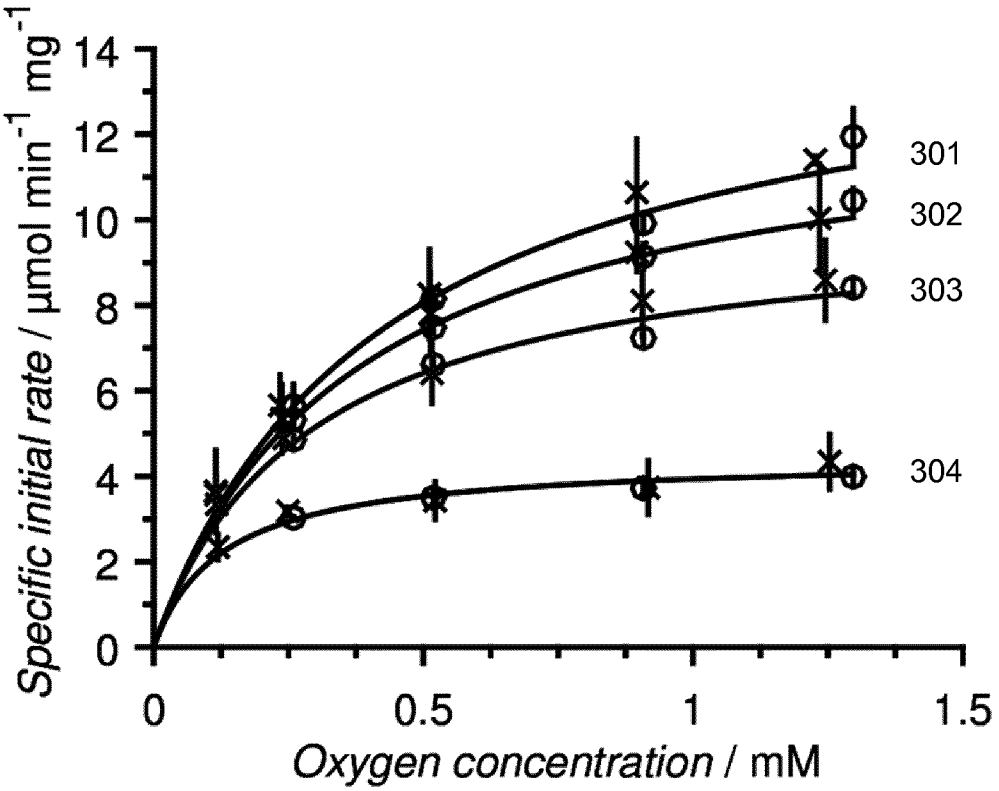


Fig. 2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14
12
10
8
6
4
2
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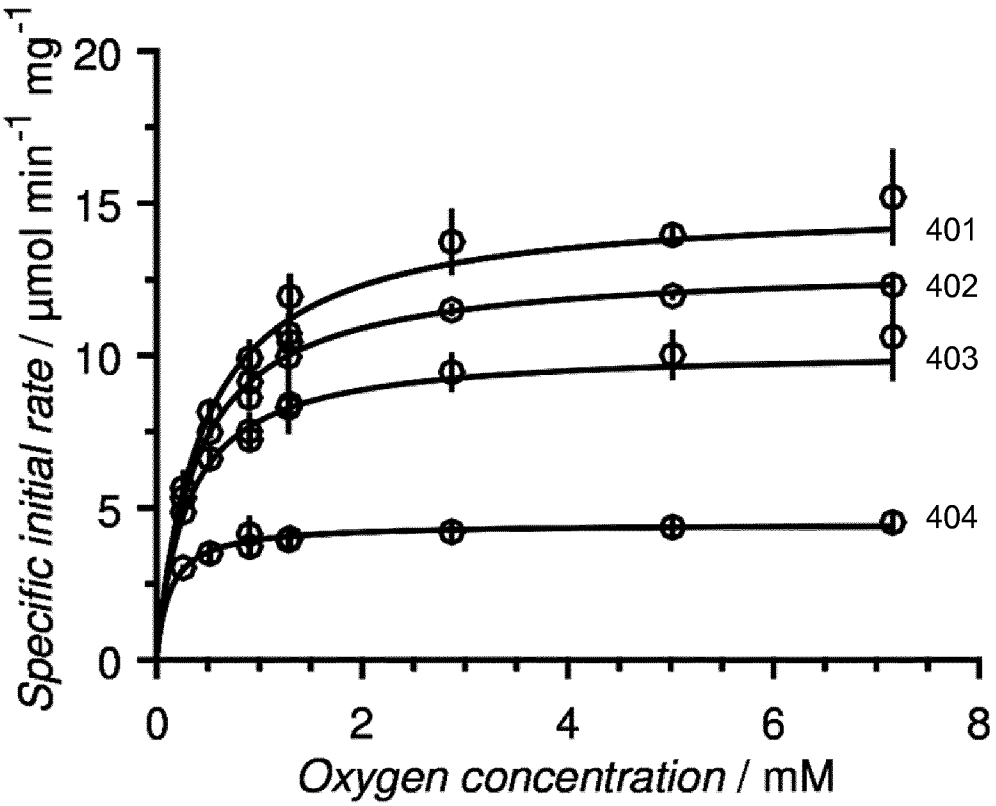
0
0.5
1
1.5
Oxygen concentration / mM
301
302
303
304

Fig. 3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15
10
5
0
0
2
4
6
8
Oxygen concentration / mM
401
402
403
404

Fig. 4

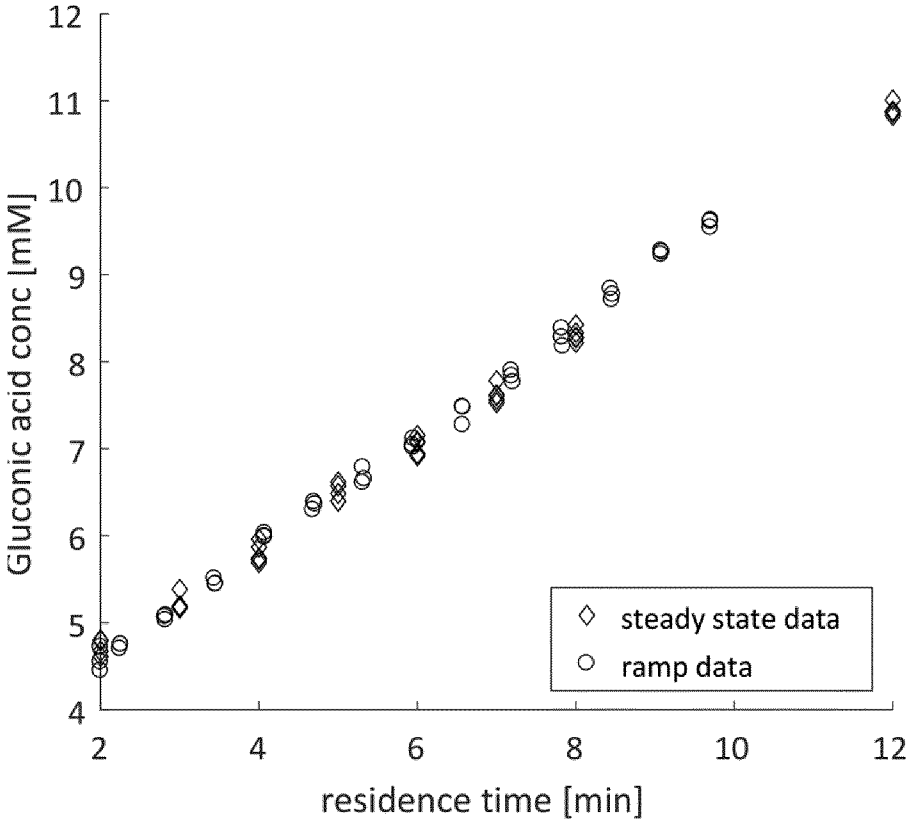
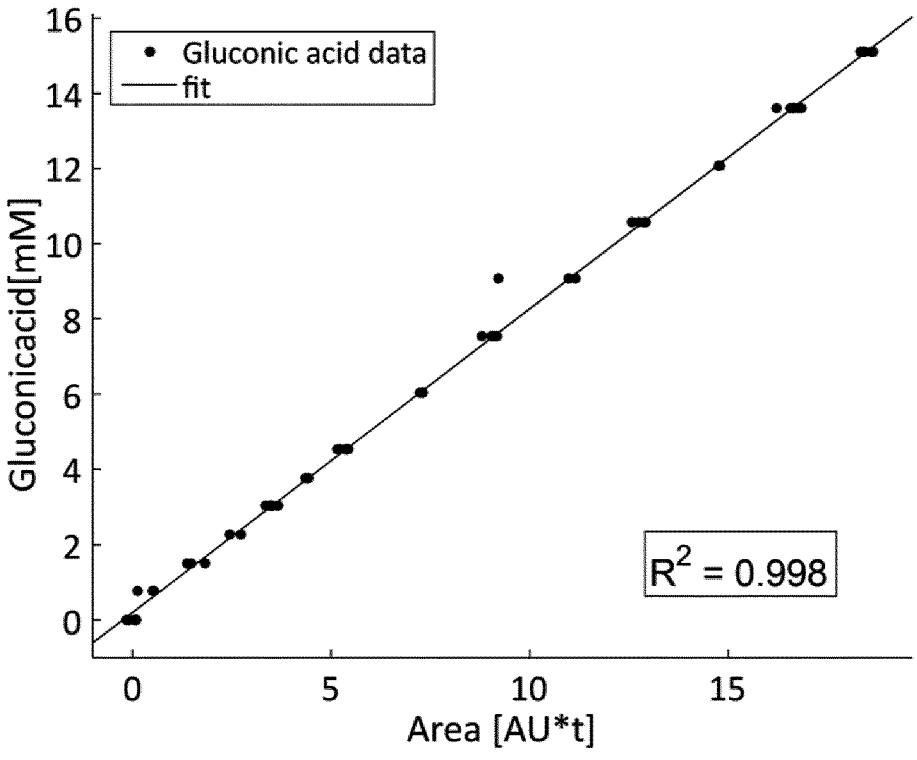

Gluconic acid conc [mM]
residence time [min]
steady state data
ramp data
12
11
10
9
8
7
6
5
4
2
4
6
8
10
12

Fig. 5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Area [AU*t]
Gluconic acid data
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12
10
8
6
4
2
0
0
5
10
15

Fig. 6

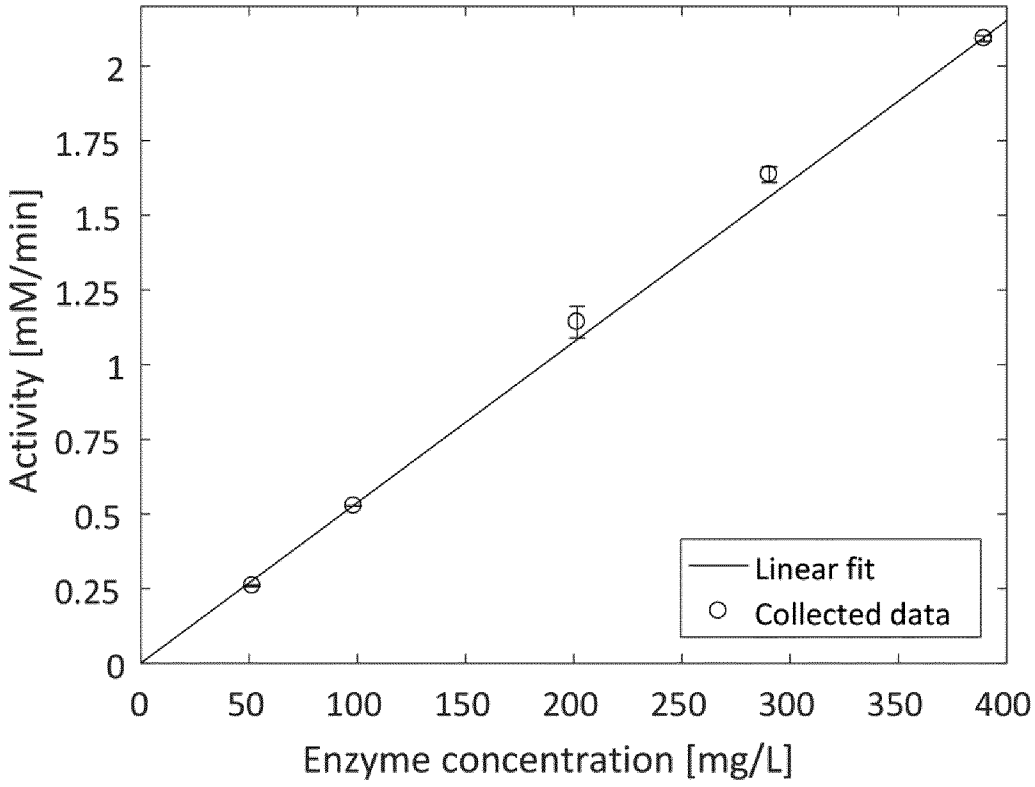
Activity [mM/min]
Enzyme concentration [mg/L]
Linear fit
Collected data
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2
0
50
100
150
200
250
300
350
400

Fig. 7

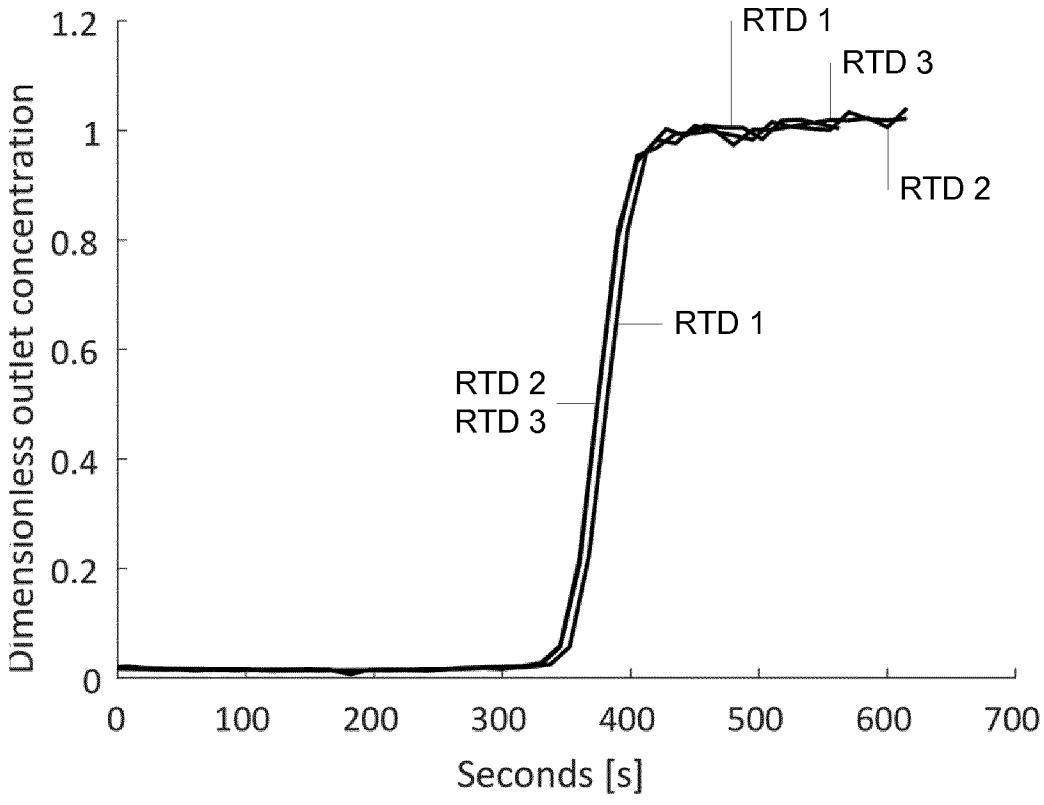
Dimensionless outlet concentration
Seconds [s]
RTD 1
RTD 3
RTD 2
RTD 1
RTD 2
RTD 3
0
0.2
0.4
0.6
0.8
1
1.2
0
100
200
300
400
500
600
700

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/059390

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01J19/00 B01J19/24 C12M1/40 C12M1/12 C12M1/36
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01J C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Ringborg Rolf ET AL: "General rights Copyright and moral rights for Application of A Microfluidic Tool for the Determination of Enzyme Kinetics", Kgs. Lyngby: Danmarks Tekniske Universitet, 1 January 2015 (2015-01-01), XP055412400, Retrieved from the Internet: URL:http://orbit.dtu.dk/files/121767170/Application_of_A_Microfluidic_Tool_for_the_Determination_of_Enzyme_Kinetics.pdf [retrieved on 2017-10-04] abstract page 48 - page 50; figure 5.4 page 64 - page 70; figure 6.1 ----- -/--	1-62

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2018	29/06/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Thomasson, Philippe

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/059390

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 538 040 A (VAPOURTEC LTD [GB]) 9 November 2016 (2016-11-09) abstract -----	1-62
A	GARCIA-OCHOA F ET AL: "Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview", BIOTECHNOLOGY ADVANCES, ELSEVIER PUBLISHING, BARKING, GB, vol. 27, no. 2, 1 March 2009 (2009-03-01), pages 153-176, XP025873361, ISSN: 0734-9750, DOI: 10.1016/J.BIOTECHADV.2008.10.006 [retrieved on 2008-11-12] abstract -----	1-62

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/059390

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2538040 A	09-11-2016	NONE	