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## Continuous screening system for inhibited enzyme catalysis: A membrane reactor approach



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**Research Article****Continuous screening system for inhibited enzyme catalysis: A membrane reactor approach**

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**Keywords:**

Screening system, enzyme membrane reactor, membrane continuous stirred tank reactor,  
cellulose hydrolysis, feedback control

**Abbreviations:**

**HTS**, High-throughput screening; **MTP**, Microtitre plate; **STR**, Stirred tank reactor; **CSTR**,  
Continuous stirred tank reactor; **MCSTR**, Membrane continuous stirred tank reactor; **DO**,  
dissolved oxygen; **OD**, optical density

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3 The screening of catalysts, substrates or conditions in the early stages of bioprocess  
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5 development requires an enormous number of experiments and is a tedious, expensive and  
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7 time consuming task. Currently available screening systems can only be operated in batch or  
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9 fed-batch mode which can lead to severe misinterpretations of screening results. For example,  
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11 catalysts that are inhibited by substrates or accumulating products will be excluded from  
12  
13 further investigations in the early stages of process development despite the fact that they  
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15 might be superior to other candidates in a different operational mode. Important and  
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17 advantageous properties such as turnover stability can also be overshadowed by product  
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19 inhibition. The aim of this study is to develop a novel screening system which enables  
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21 continuous feeding of substrates and continuous removal of products. A prototype based on  
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23 the membrane reactor concept was designed and operated for a model reaction, the hydrolysis  
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25 of cellulose.  
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## 1. Introduction

### 1.1 State of the art

The search for a suited biocatalyst, its optimum conditions and substrates or novel products is the first step in each bioprocess development. This is typically achieved in a large number of parallel experiments in micro- to millilitre scale (high-throughput screening, HTS). This high degree of parallelism, however, is achieved at the cost of largely uncontrolled process operation. Although sometimes very large scale-up ratios are used during the development of new biotechnological processes, a scale-up ratio of about 1:10 is taken as the upper acceptable limit [1, 2]. The following four scales are typically mentioned: 50-1000 mL (shake-flasks scale), 5-20 L (bench scale), 50-5000 L (pilot plant scale) and 10-1000 m<sup>3</sup> (production scale) [1]. Shake flasks are used typically for experiments in the scale up to 1000 mL. Such experiments are quite inexpensive and simple, however significant problems like insufficient oxygen supply, inefficient mixing or inability to continuously monitor them are often mentioned [3]. For this reason many producers as well as research groups attempt to develop new screening systems, which are able to overcome the given drawbacks. Table 1 summarises commercially available and recently developed screenings systems (above MTP scale) up to bench scale. The main focus is on STR based screening systems which are typically favoured for secondary screening, since the large majority of industrial fermentations is carried out in stirred bioreactors.

As seen above, currently available systems can mainly be operated in batch or fed-batch mode, which especially for inhibited reactions can lead to severe misinterpretations of screening results. For example, catalysts that are inhibited by accumulating products or high initial substrate concentrations will be excluded from further investigations in the early stage of process design, despite the fact that they might be superior to other candidates when operated in a different mode. Some attempts have been made to develop continuous screening

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3 reactors [4], but such systems do not contain a filtration module so application is limited to  
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5 whole cell transformations and immobilised enzymes. However, this problem can be  
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7 eliminated by using an ultrafiltration membrane which retains dissolved enzymes. This  
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9 project thus aims at developing a novel screening system which enables continuous feeding of  
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11 substrates and continuous removal of products. It will thus, e.g., reduce the amount of  
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13 unwanted by-products, or increase product yield or reaction rate. In addition, important  
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15 process parameters like residence time and volumetric power input shall be determined and  
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17 feed-back controlled if required to facilitate safe scale-up of results to bench-scale. Table 2  
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19 gives a survey of commercially available and developed membrane-based reactor systems for  
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21 enzymatic reactions.  
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27 Although a lot of membrane-based reactor systems have been developed, to our knowledge  
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29 only one attempt was made to bring to market an enzyme membrane reactor with continuous  
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31 operation mode. Unfortunately this system is not available anymore, since the company  
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33 (Jülich Fine Chemicals GmbH) ceased to exist in 2005. Common for all developed systems is  
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35 that they do not work in parallel. Also, often the main parameters like residence time or pH  
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37 are not controlled. Another aspect is the costs of developed systems, which even for small  
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39 scale EMR could be quite high [12]. The systems which are summarised in Table 2 can be  
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41 divided into 3 categories according to the form of substrate-enzyme contact [29]: those with  
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43 direct S-E contact, those with indirect S-E contact and two-phase membrane reactors. Fig. 1  
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45 shows typical designs for each category.  
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51 From the above it is obvious that the membrane can fulfil very different tasks: it can be used  
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53 to separate the enzyme from the product (A), for bubble free aeration (C), provide a contact  
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55 surface/interface for two-phase mass transfer (E), etc. No single system can satisfy all needs  
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57 and a compromise between functionality or versatility and economics has to be made. Thus,  
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59 this work initially focuses on direct S-E contact applications.  
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## 1.2 Design of a novel screening system for potentially inhibited biocatalysis

With regards to the mentioned shortcomings of currently available systems, the following requirements were specified. The novel concept must:

- be suited for non-immobilised enzymes
- not exceed a working volume of 100 mL
- enable scale-up to bench-scale
- enable monitoring and control of: T, pH, DO, OD, Redox and residence time  $\tau$
- enable parallel operation of several reactors (>2)
- be low priced

Besides monitoring and if possible feed-back control of pH, temperature, etc., the main parameter that needs to be reliably controlled in a continuous MCSTR is residence time or, in other words, membrane permeability. Transmembrane flux and thus product removal can be achieved by either permeate suction or by pressure increase on the feed side. The former necessitates the use of an adjustable permeate pump, and the transmembrane pressure is limited to 1 bar atmospheric pressure which might not be enough to ensure a satisfactory flux throughout the duration of the process. In the latter option, no permeate pump but a control valve and a pressure source are required, and higher transmembrane pressures can be utilised. This, on the other hand, necessitates the use of pressure resistant sensors and equipment. In order to maintain the required membrane permeability, the formation of deposits on the membrane surface must be limited. This can be achieved by shear and lift forces, e.g. either accomplished in external cross-flow filtration or by rotating devices close to the surface. External filtration requires an additional pump and provides a larger surface onto which enzymes, substrate and products can adsorb, hence, in-situ filtration was selected. Fig. 2 shows P&I diagrams of two such in-situ MCSTRs in which permeation is achieved by increased feed pressure. If oxygen is required for the reaction, option A must be used, while option B will suffice in all other cases. Since less than 20 % of industrial applications use

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3 oxidoreductases [30], option B is chosen for this study. Feedback control of the residence  
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5 time is achieved by monitoring the flow rate and adjusting the feed pressure.  
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### 10 1.3 Choice of a suited model reaction 11

12 For a first feasibility study of the reactor concept, a model reaction is applied. It was decided  
13 to use a hydrolysis since 72 % of industrial enzyme catalysis is performed with hydrolases  
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15 [30]. The hydrolysis of cellulose was found to be particularly suited for the proof of concept  
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17 of the continuous screening system since:  
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- 20 • the reaction is well known and described
- 21
- 22 • it is widely applied in the industries of the energy sector, food and chemicals [31]
- 23
- 24 • it is strongly inhibited by its products
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- 26 • it cannot be performed with immobilised enzymes
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31 The latter two render the use of an MCSTR an attractive option. The reaction proceeds in four  
32 steps (see. Fig. 3). Its products glucose and cellobiose strongly inhibit the last two steps.  
33

34 While cellobiose inhibition can be reduced by the addition of further cellobiase (i.e.  $\beta$ -  
35 *glucosidase*), this enzyme itself is inhibited by the presence of glucose, which can only be  
36 prevented by in-situ product removal [32].  
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## 46 2. Materials and methods 47

### 48 2.1 Implementation of the screening system 49

50 The main components are: the membrane reactor, a mixing device, a thermostat, a control  
51 valve and a flow meter. Two stirred dead-end cells (XFUF-047, Millipore Corporation) with a  
52 working volume of 86/89,8 mL (batch/continuous) and a diameter of 47 mm were used as the  
53 membrane reactors. A flat-sheet membrane (eff. surface 14.7 cm<sup>2</sup>) is placed at the bottom.  
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58 With this surface to volume ratio, residence times in the range of 2-24 h can be realised  
59 assuming typical fluxes between 2.5-30 L/(m<sup>2</sup>h). The cell is mixed by a magnetic stirrer (2  
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3 MAG MIX 1) that enables accurate stirrer speed control in the range between 100 bis 2000  
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5  $\text{min}^{-1}$ . Retentate pressure was controlled with a pressure regulator (MPPE-3, Festo AG,  $\pm 20$   
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7 mbar accuracy). The MCSTRs were placed in a water bath that was temperature controlled  
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9 (thermostat D1, Thermo Haake GmbH). An electronic precision balance (ALT 310, Kern &  
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11 Sohn GmbH) was used to measure the permeate flow. Data were logged onto a computer with  
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13 a frequency of 4 Hz. To enable robust control, values were averaged over 25 s (100 values).  
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15 The use of a volumetric flow meter would be advantageous because of robustness, unlimited  
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17 operation time, and simpler integration into a parallel reactor set-up, but flow meters in the  
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19 required low measuring range of around 5 mL/h are about twice as expensive. They would,  
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21 however, just change the controller settings and not the idea of the control scheme (see 3.1).  
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23 pH was measured in the permeate. Visual Designer™ (Version 4.0) was used to modulate and  
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25 optimise the PI/PID controller settings.  
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## 34 2.2 Choice of enzyme, substrate and membrane

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36 Cellulase from *Trichoderma reesei* (Sigma-Aldrich, C8546) was used. This enzyme contains  
37  
38 three constituents: *exocellulase*, *endocellulase* and  *$\beta$ -glucosidase* (cellobiase). Their  
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40 molecular weight can range from 23-105 kDa [34]. Hence, an ultrafiltration membrane with a  
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42 MWCO of 10 kDa (UP010, PES, Microdyn-Nadir GmbH, Germany) was used to retain the  
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44 enzymeconsortium. Even a membrane with a higher MWCO of 30 kDa was shown to retain  
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46 around 90 %  *$\beta$ -Xylanase* (MW 20 kDa, [35]). The molecular weight of the products is 180  
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48 and 342 Da, respectively, so it is safe to assume that the products pass the membrane  
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50 unhindered.  *$\alpha$ -cellulose* (Sigma-Aldrich, C8002) is used as the substrate.  
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## 58 2.3 Analytical methods

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60 The surrogate concentration of reduced sugars was measured using refractometry (DD-7,  
precision  $\pm 0.005$  % [Brix], ATAGO Co., Ltd.) which was calibrated with a glucose standard.

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3 From this concentration, cellulose conversion was calculated. Samples were taken from the  
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5 permeate.  
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#### 10 2.4 Enzyme activity

11 The required amount of enzymes was dosed in units/L. Here, one unit was defined as the  
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13 amount of enzymes that is required to yield 1  $\mu\text{mol/h}$  glucose from cellulose at  $T = 37\text{ }^\circ\text{C}$  and  
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15  $\text{pH} = 5$  over a period of 2 h. Enzyme activity was performed in accordance with the *Sigma-*  
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17 *Aldrich Control Test Procedure* [36].  
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### 25 3. Results and Discussion

#### 26 3.1 Feedback Control Concept

27 Residence time, i.e. flow rate control is carried out with the feed pressure as the actuating  
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29 variable. Since the systems' response to a step change showed a typical  $\text{PT}_n$ -behaviour,  
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31 closed-loop control could be achieved with a PI or PID controller. Therefore, the system  
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33 response was initially approximated with  $\text{PT}_1\text{T}_0$  and  $\text{PT}_n$  models. Different settings according  
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35 to Ziegler and Nichols [37], Kuhn [38], Chien et al. [39] and Schwarze [40] were then  
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37 assessed with respect to their disturbance reaction. Fig. 4 shows an example of this procedure  
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39 for Kuhn [38] settings. During the first 200 s, the system was not controlled and the curve just  
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41 represents the system response. Feedback control is then switched on, and the emerging curve  
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43 is a measure of the set value behaviour. After 670 s, a defined and reproducible disturbance  
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45 was induced and again the system response evaluated. Thus, an evaluation of different  
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47 controller settings could be performed. The comparison between different controller settings  
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49 showed for example, that with the settings according to Ziegler and Nichols [37] as well as  
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51 Chien et al. [39] the system responded well to disturbances, but it takes a long time to reach  
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53 the set values. It could also be detected, that in all cases the PID settings led to faster  
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55 attainment of the set values. However, when disturbances were introduced, the fluctuations  
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3 and the time required to attain the set values were minimised when PI settings were used.  
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5 After the comparison of different PI settings according to Ziegler and Nichols [37], Kuhn  
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7 [38], Chien et al. [39] and Schwarze [40], the settings according to Kuhn [38] were found to  
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9 be the best in terms of their disturbance behaviour and set values behaviour. For all following  
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11 experiments these settings were used.  
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15 Fig. 5 shows results of a long-term study which should primarily prove the control concept's  
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17 ability to keep the flow and thus the residence time at the desired value (e.g. 10 mL/h and 9 h,  
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19 respectively) with an acceptable accuracy over a longer period and with potential membrane  
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21 fouling.  
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25 As can be seen from Fig. 5, a stable flow and thus residence time feedback control could be  
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27 implemented using controller settings acc. to Kuhn. The volumetric flow rate was 9.998 mL/h  
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29 on average over 20 h and a standard deviation value of 0.228 mL/h was calculated based on  
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31 the assumption of a Gaussian distribution of the data shown in figure 5. The pressure increase  
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33 after approximately 16 hours can be explained by membrane fouling (e.g. successive closure  
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35 of pores until the local flux exceeds the critical flux) which occurs during filtration of  
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37 biological suspensions even under cross-flow conditions, i.e. continuous removal of  
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39 accumulations from the membrane surface [41]. Visual inspection showed that the deposit,  
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41 however, was so small that the homogeneity of the reactor content was not affected.  
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### 48 3.2 Parallel Batch Operation

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50 Fig. 6 shows results of two parallel batch experiments. The hydrolysis was carried out at a  
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52 temperature of 40°C with a sodium acetate buffer (pH = 4.66). These conditions were selected  
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54 according to manufacturer's recommendations which do not mean that they are necessarily  
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56 optimal. Enzyme concentration used was 200 mg/L (measured enzyme activity was 5.6 u/mg)  
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58 which enabled comparison with published results. 2 mL samples were taken from the  
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60 permeate and immediately returned after analysis in order to keep the reactor volume and

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3 enzyme concentration constant. Conversion was calculated under the assumption of an ideally  
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5 mixed reactor (Eq. 1):  
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$$10 \quad \text{Conversion}(t) = \frac{c_{\text{Sample}}(t)}{c_{S,0}} \cdot 100\% \quad (1)$$

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17 It can be seen that there is a good fit between the obtained data and the data published,  
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19 measured under identical conditions, despite the fact that enzyme concentration cannot be  
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21 compared exactly since dosing in Gan et al. [28] was done in mg/L (200 mg/L) and the  
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23 enzyme activity was not mentioned. Because a similar time dependent conversion was  
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25 observed for the same concentration, it may be stated that the activity of our enzyme was  
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27 comparable to that of Gan's. Fig. 6 also shows the parallelism of both reactors. Deviations are  
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29 less than 8 % (to the averaged values) at all times. Possible reasons for the decreasing rate of  
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31 conversion include substrate depletion, product inhibition and enzyme deactivation. Gan et al.  
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33 [28], too, observed such a decrease although the data chosen for comparison here almost  
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35 suggest a linear progress.  
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### 43 3.3 Continuous vs. Batch Operation

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45 Fig. 7 shows a comparison of cellulose conversion in batch and continuous mode under  
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47 otherwise equivalent conditions (enzyme and substrate concentration, temperature, pH, power  
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49 input). In continuous operation, the insoluble substrate and enzymes were added to the reactor  
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51 only at the beginning. Then buffer was added continuously and product solution withdrawn at  
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53 the same rate during the entire reaction period. The hydrolysed sugars were removed from the  
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55 reactor while substrate and enzymes were retained. The desired residence time of 6 h was  
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57 controlled with an accuracy of  $\pm 1$  % over the whole duration of the experiment (data not  
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showed). Similar to the batch operation, the conversion was calculated under the assumption of an ideally mixed reactor (Eq. 2):

$$Conversion(t) = \frac{V_{Collected}(t) \cdot \bar{c}(t) + V_{Sample} \cdot c_{Sample}(t) + V_{Reactor} \cdot c_{Sample}(t)}{V_{Reactor} \cdot c_{S,0}} \cdot 100\% \quad (2)$$

With a deviation of < 2.5 % (to the averaged values), the reproducibility of continuous experiments is very high. It is obvious that continuous operation increases cellulose conversion and reaction rate by around 60 % in comparison to batch. After 60 h, 70.6 % of the initially provided substrate have been converted in the continuous run, while at the same time, only 43.5 % were achieved in batch mode. Or, in other words, to reach the same conversion, an increased reaction time is required (already more than doubled for 40% conversion). Since all operating conditions were the same, this increase in reaction rate can only be attributed to the product inhibition that dominates in the batch run. However, since reaction rate depends on substrate concentration, conversion slows down in the continuous mode, too.

Fig. 8. shows the corresponding product concentration evolution for Fig. 7 in continuous and batch operation mode. Clearly, the reaction inhibition in case of batch operation is strongly pronounced due to the high concentration of glucose and cellobiose (which is 11 times higher than in continuous operation after 60 h). In the case of continuous mode, operation at a constant residence time decreases product concentration. This also indicates one of the biggest drawbacks of continuous operation: in production processes, the product concentration in the permeate flow could be too low for directly further usage, e.g. for ethanol production, a glucose concentration between 8 and 10 % has been suggested [31].

In comparison with published data, a significantly higher yield was achieved here. The residence time was not given, but under otherwise similar conditions, Gan et al. [28] had

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3 reached max. 53 % after 48 h of continuous operation, while in this work, 63.5 % were  
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5 obtained (see Fig. 7).  
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#### 10 **4. Concluding remarks**

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12 Currently available screening systems have several drawbacks, e.g. the fact that they cannot  
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14 be operated continuously. In order to release the potential of possibly product inhibited  
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16 catalysts, a screening reactor concept based on a membrane CSTR was conceived and  
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18 constructed. The concept's potential was shown using the enzymatic hydrolysis of cellulose as  
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20 a model reaction. In this feasibility study, some imperative key features were attested: The  
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22 novel system (1) enables screening of product or substrate inhibited enzymes which would  
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24 have been excluded from future studies in normal (batch) screening systems, (2) can be  
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26 operated at a controlled residence time ( $\pm 1$  % accuracy), (3) shows good agreement in parallel  
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28 experiments, and (4) yields reproducible results.  
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41  
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51 The authors have declared no conflict of interest.  
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#### 55 **List of symbols**

56			
57	F	Flow rate	[m <sup>3</sup> /s]
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59	P	Pressure	[Pa]
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	T	Temperature	[K]

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t	Time	[s]
$\tau$	Residence time	[s]
n	Rotation speed	[s <sup>-1</sup> ]
c	Concentration	[kg/m <sup>3</sup> ]
V	Volume	[m <sup>3</sup> ]

**Subscripts**

S	Substrate
E	Enzyme
0	Initial

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3 Figure 1: MCSTR configurations: A) with direct S-E contact and in-situ filtration; B) with direct S-E contact and  
4 external filtration; C) with direct S-E contact and membrane aeration; D) MCSTR with indirect S-E contact and  
5 external filtration (dialysis); E) two-phase MCSTR with external circulation (membrane contactor); ●: enzyme;  
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7 S: substrate; P: product; MC: Membrane Contactor; MAU: Membrane Aeration Unit  
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13 Figure 2: In-situ MCSTR concepts: A) with oxygen supply; B) without oxygen supply; ● enzymes; ○ gas  
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15 bubbles; S: substrate; P: product  
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19 Figure 3: Suggested reaction mechanism of cellulose hydrolysis with a cellulase complex;  $C_1$ : Exocellulase;  $C_X$ :  
20 Endocellulase (adapted from [33])  
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25 Figure 4: Example of a step response: PI and PID controller acc. to Kuhn [38], set value and disturbance  
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27 behaviour for different controller settings  
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31 Figure 5: Volumetric flow rate and retentate pressure development,  $c_{S,0} = 25 \text{ g/L}$ ,  $T = 40 \text{ °C}$  (PI controller acc. to  
32 Kuhn [38])  
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36 Figure 6: Cellulose conversion during parallel batch runs,  $c_E = 1120 \text{ U/L}$ ,  $c_{S,0} = 25 \text{ g/L}$ ,  $\tau = 6 \text{ h}$ ,  $T = 40 \text{ °C}$ ,  $\text{pH} =$   
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38  $4.66$ ,  $n = 100 \text{ min}^{-1}$   
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42 Figure 7: Cellulose conversion during batch and continuous operation,  $c_E = 1120 \text{ U/L}$ ,  $c_{S,0} = 25 \text{ g/L}$ ,  $\tau = 6 \text{ h}$ ,  $T =$   
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44  $40 \text{ °C}$ ,  $\text{pH} = 4.66$ ,  $n = 100 \text{ min}^{-1}$   
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49 Figure 8: Concentration profile for products (glucose and cellobiose) during batch and continuous operation,  $c_E =$   
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51  $1120 \text{ U/L}$ ,  $c_{S,0} = 25 \text{ g/L}$ ,  $\tau = 6 \text{ h}$ ,  $T = 40 \text{ °C}$ ,  $\text{pH} = 4.66$ ,  $n = 100 \text{ min}^{-1}$   
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Table 1: Overview of commercially available and prototype screening systems

System/ Ref.	Volume/Work. Volume [mL]	Instrumentation/ Control	Number of parallel reactors	Aeration	Operational mode
<b>Shake Flasks</b>					
Sensolux/ Sartorius AG	125-1000	pH, DO	9	none	Batch
SFR/PreSens GmbH	Up to 1000	pH, DO	9	none	Batch
[4]	250	F/F	4	gas sparging	Fed-batch
[5]	500/100	pH, P/pH, F	9	none	Fed-batch
<b>Bubble Columns</b>					
[6]	5/2	P, F	25	gas sparging	Fed-batch
[7]	500/200	pH, F /pH	12	gas sparging	Fed-batch
<b>STR</b>					
Cellstation/ Fluorometrix	/35	pH, DO, OD/ T, n	12	none	Batch
Xplorer/ Bioxplore (HEL Ltd.)	/30-100	T, pH, DO, F/ T, pH, DO, n	8	4-gas mixing	Fed-batch
Explorer/ Medicell Oy	/100-500	T, pH, DO, OD, F/ T, pH, DO, n, F	15	3-gas mixing	Fed-batch
DASGIP AG Bioblock	/150-4000	T, pH, DO, OD, F, Foam/ T, pH, DO, n, Foam, F	16	4-gas mixing	Fed-batch
Biostat Qplus /Sartorius AG	/500-1 000	T, pH, DO, F, Foam/ T, pH, DO, n, Foam, F	12	4-gas mixing	Fed-batch
Multifors/ Infors AG	/500-1000	T, pH, DO, F, Foam/ T, pH, DO, n, Foam, F	6	4-gas mixing	Fed-batch
Biostat B-DCU- II/Sartorius AG	/500 - 10 000	T, pH, DO, F, Foam/ T, pH, DO, n, Foam, F	6	4-gas mixing	Fed-batch
[8]	/2	pH, DO, OD/ DO(on-off), n	24	gas sparging	Batch
[9]	/12	T, pH, DO, OD, F/ T, pH, F, n	48	gas sparging	Batch
[10]	/30	pH, DO/ T, n	12	2-gas mixing	Batch
[11]	/100	T, pH, DO, OD, F/T, n	4	gas sparging	Batch

Table 2: Overview of commercially available and developed membrane based reactor systems

System/ Ref.	Work. Volume [mL]	Instrumentation /Control	Number of parallel reactors	Number of Phases/Remark	Operational mode
EMR/ JFC GmbH	10	P	1	1	Continuous
Celline/ Sartorius AG	350-1000	-	1	1/Disposable	Batch
[12]	0,2	/F	1	1	Continuous
[13]	10	/T, n	1	1	Continuous
[14]	10	/T, n	1	1	Continuous
[15]	18	T, P, F/T	1	1/High-pressure reactor	Continuous
[16]	25	P, F/T, F	1	1/Aerated	Continuous
[17]	40	/T, n	1	1/Water sorption with mol. sieves	Batch
[15]	45	T, P, F/T	1	1/High-pressure reactor	Continuous
[18]	50	/T, n	1	1	Continuous
[19]	100	/T, n	1	2	Batch
[20]	~60	P/T	1	2/Tubular reactor	Pseudo - continuous
[21]	110	/T, n	1	1/Pevaporation	Continuous
[22]	120	/T, n	1	1/ Pseudo-homogeneously	Continuous
[23]	500	/T	1	1/Membrane extraction	Continuous
[24]	~600	P/T, n	1	1	Continuous
[25]	600	/T	1	1	Continuous
[26]	600	P/T, pH	1	2	Batch
[27]	1500	T, F/T, pH	1	1	Continuous
[28]	2500	T, pH, F/T, n	1	2/El. membrane cleaning	Pseudo-continuous

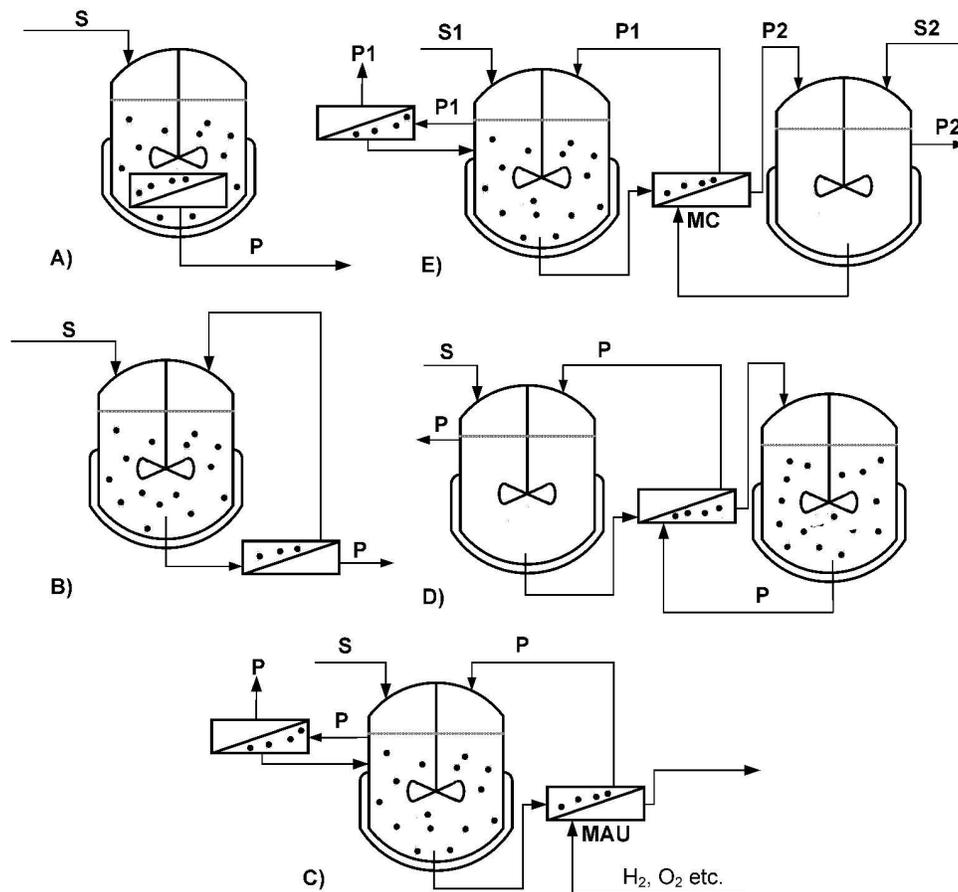


Figure 1: MCSTR configurations: A) with direct S-E contact and in-situ filtration; B) with direct S-E contact and external filtration; C) with direct S-E contact and membrane aeration; D) MCSTR with indirect S-E contact and external filtration (dialysis); E) two-phase MCSTR with external circulation (membrane contactor); ●: enzyme; S: substrate; P: product; MC: Membrane Contactor; MAU: Membrane Aeration Unit

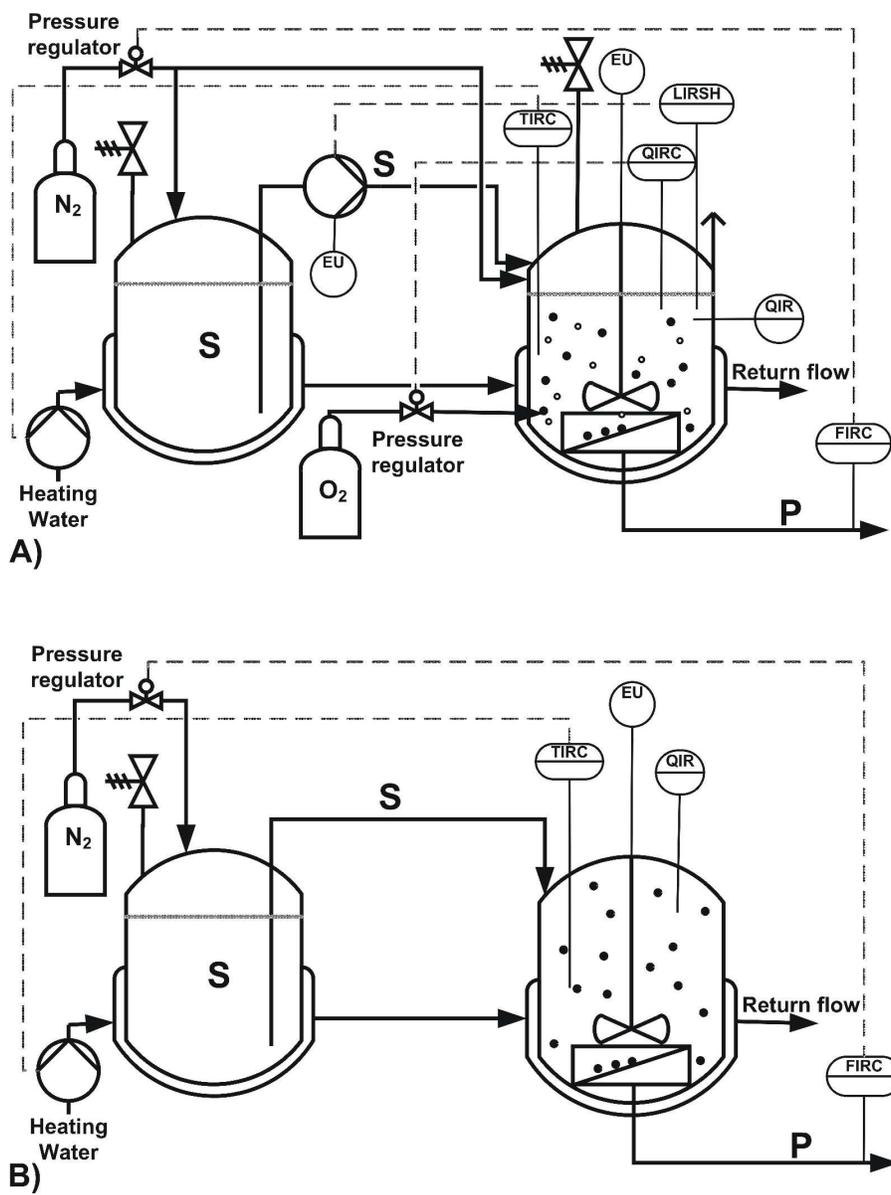


Figure 2: In-situ MCSTR concepts: A) with oxygen supply; B) without oxygen supply; • enzymes; ○ gas bubbles; S: substrate; P: product  
852x1129mm (144 x 144 DPI)

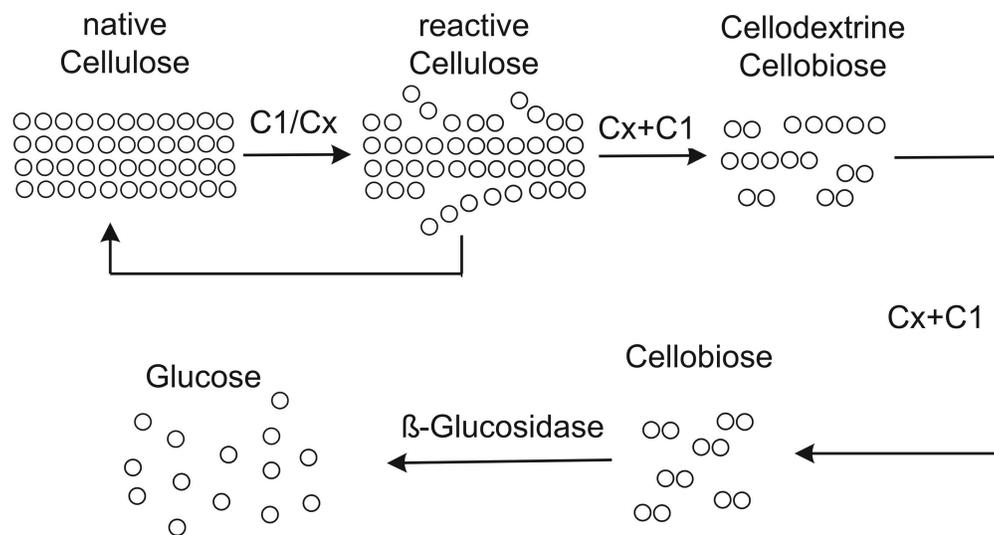


Figure 3: Suggested reaction mechanism of cellulose hydrolysis with a cellulase complex; C1: Exocellulase; CX: Endocellulase (adapted from [33])  
116x62mm (600 x 600 DPI)

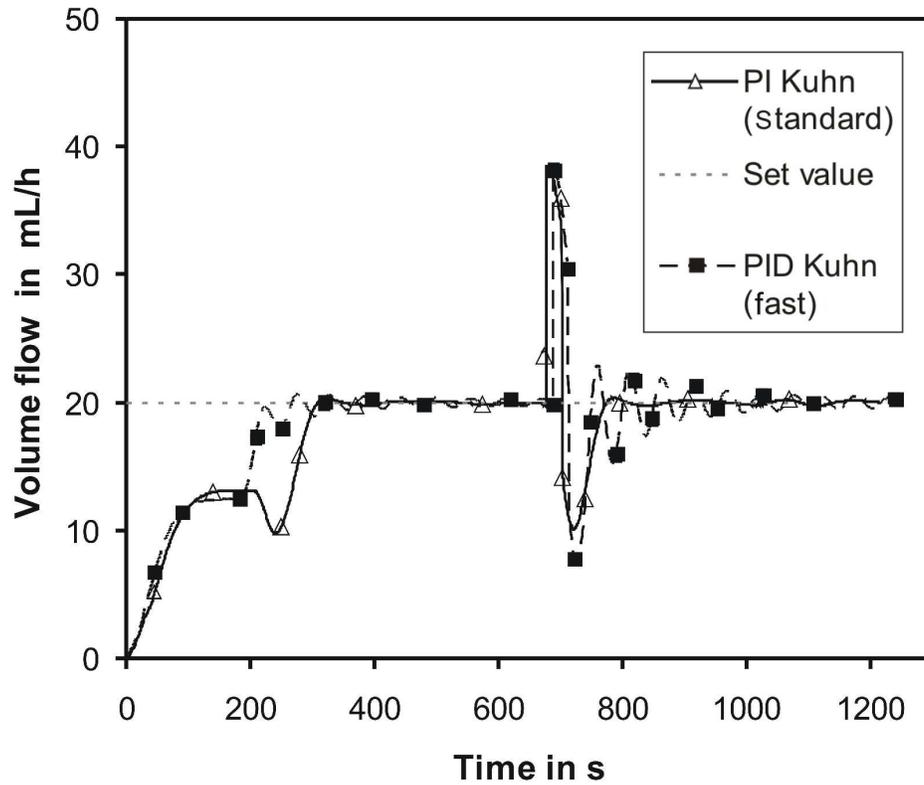


Figure 4: Example of a step response: PI and PID controller acc. to Kuhn [38], set value and disturbance behaviour for different controller settings  
913x765mm (144 x 144 DPI)

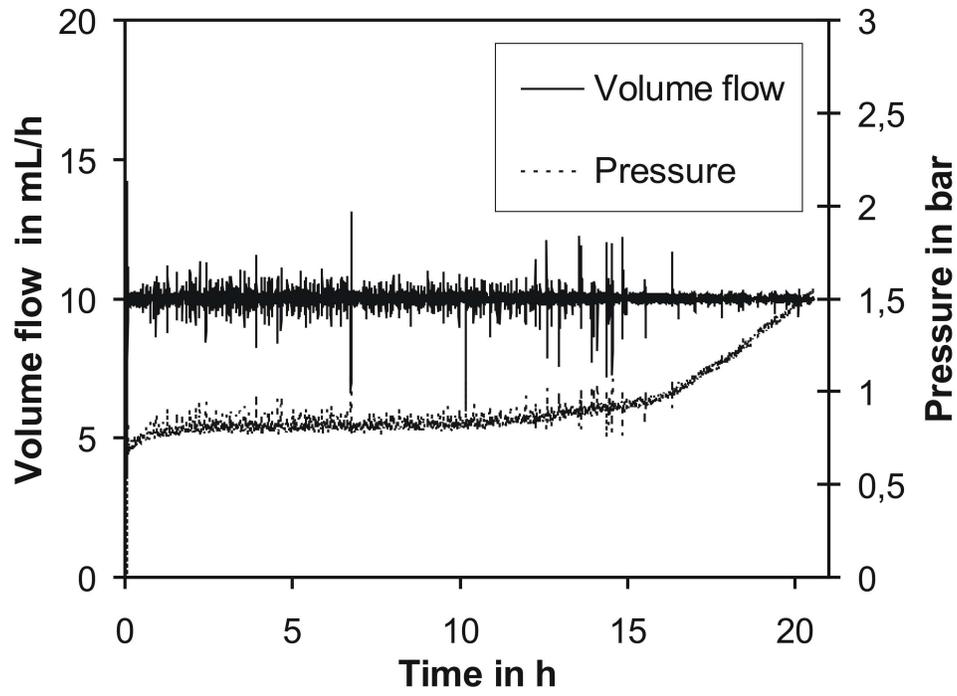


Figure 5: Volumetric flow rate and retentate pressure development,  $c_{S,0} = 25 \text{ g/L}$ ,  $T = 40 \text{ }^\circ\text{C}$  (PI controller acc. to Kuhn [38])  
122x89mm (600 x 600 DPI)

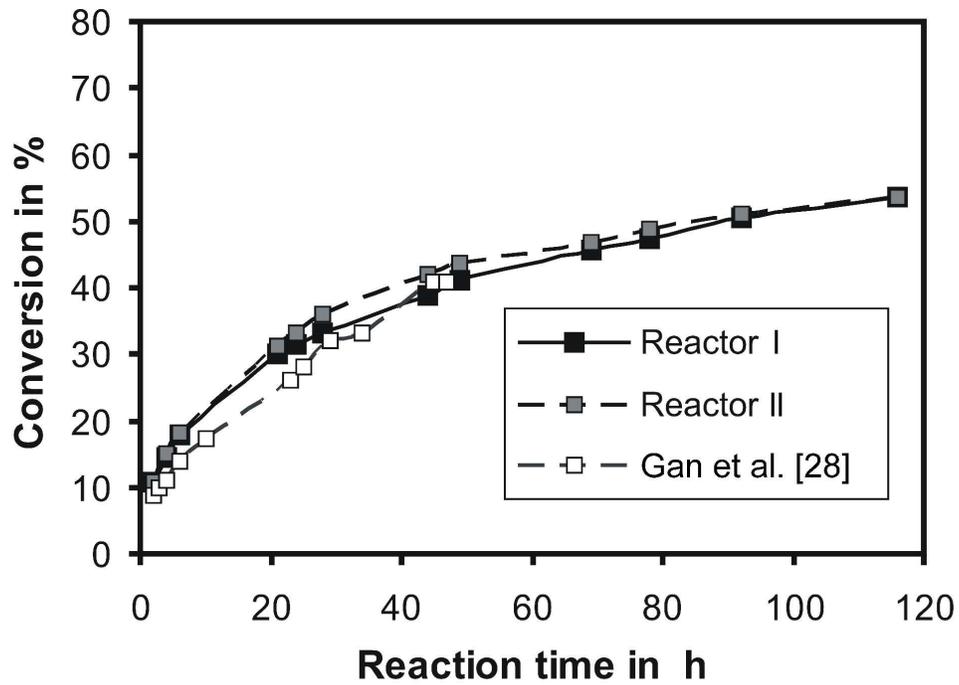


Figure 6: Cellulose conversion during parallel batch runs,  $c_E = 1120 \text{ U/L}$ ,  $c_{S,0} = 25 \text{ g/L}$ ,  $\tau = 6 \text{ h}$ ,  $T = 40 \text{ }^\circ\text{C}$ ,  $\text{pH} = 4.66$ ,  $n = 100 \text{ min}^{-1}$   
1048x775mm (144 x 144 DPI)

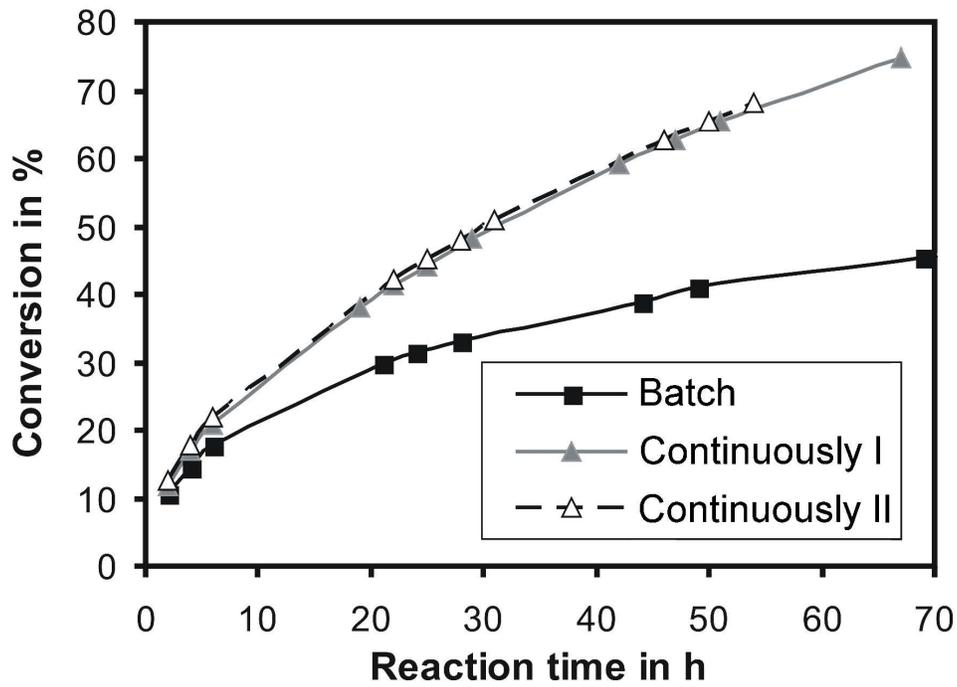


Figure 7: Cellulose conversion during batch and continuous operation,  $c_E = 1120 \text{ U/L}$ ,  $c_{S,0} = 25 \text{ g/L}$ ,  $\tau = 6 \text{ h}$ ,  $T = 40 \text{ }^\circ\text{C}$ ,  $\text{pH} = 4.66$ ,  $n = 100 \text{ min}^{-1}$   
652x486mm (144 x 144 DPI)

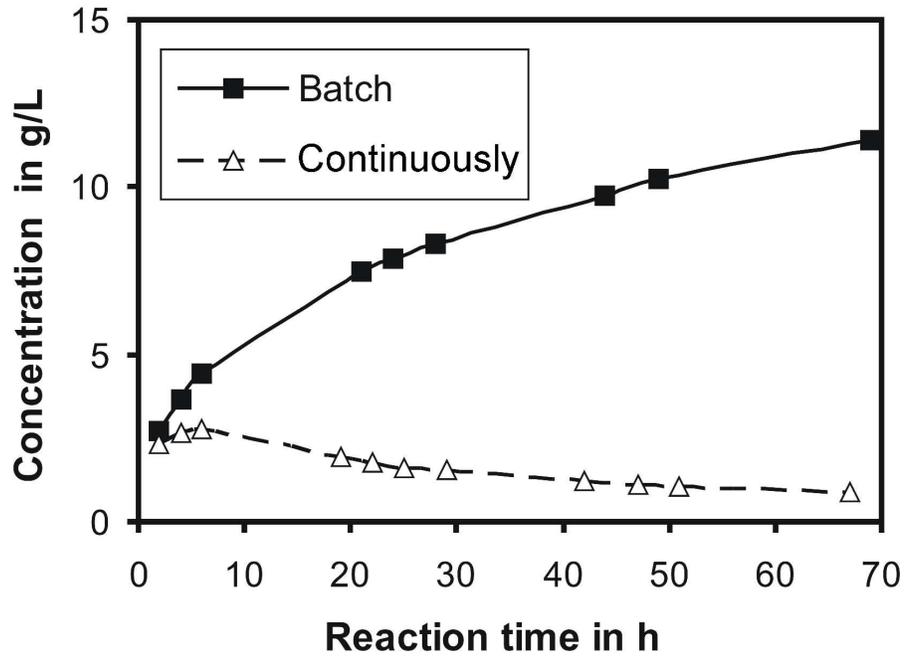


Figure 8: Concentration profile for products (glucose and cellobiose) during batch and continuous operation,  $c_E = 1120$  U/L,  $c_{S,0} = 25$  g/L,  $\tau = 6$  h,  $T = 40$  °C,  $\text{pH} = 4.66$ ,  $n = 100$  min<sup>-1</sup>  
654x483mm (144 x 144 DPI)