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Process Investigations for Development of an Advanced Bioreactor System for Thermophilic H_2 Fermentations

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1 Introduction

Hydrogen production via extreme thermophilic fermentation (70°C) of biomass has several advantages above mesophilic fermentations. The outstanding characteristic is the high hydrogen yield, which can reach nearly the theoretical amount of 4 moles H_2 per mole of hexose. Another advantage is the repression of methanogenic microorganisms, which consume the produced hydrogen, and the elimination of pathogenic organisms. Nevertheless there are several problems, which have to be reconsidered in order to set up a successful industrial process, like the product inhibition by the generated hydrogen, the rather low cell densities and the sensitivity towards high substrate as well as metabolite concentrations. The hydrogen partial pressure is conventionally reduced by applying stripping with inert gas [1, 2], which is a non economical method for bigger scales. To overcome hydrogen inhibition, different methods as vigorous stirring or recirculation have been investigated before [3]. In this work the effect of underpressure in order to reduce hydrogen partial pressure as well as the insertion of porous solid particles was studied, which are supposed to enforce bubble initiation and to drive the release of hydrogen from the liquid. This work is part of [4], an Integrated Project supported by the European Commission under the 6th Framework Program.

2 Materials and Methods

2.1 Organism and medium

The strain Caldicellulosiruptor saccharolyticus (DSM 8903) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

The medium used for the experiments contained (per L): 5 g glucose, 0.9 g NH_4Cl, 0.75 g KH_2PO_4, 1.49 g K_2HPO_4, 1 g yeast extract, 0.4 g $MgCl_2 \cdot 6H_2O$, 0.6 g L-cysteine, 2.5 mg $FeCl_3 \cdot 6H_2O$ and 1 ml trace element solution, containing per L: 13 ml HCl 6M, 1.5 g $FeCl_2 \cdot 4H_2O$, 70 mg $ZnCl_2$, 100 mg $MnCl_2 \cdot 4H_2O$, 6 mg H_3BO_3, 190 mg $CoCl_2 \cdot 6H_2O$, 2 mg $CuCl_2 \cdot 2H_2O$, 24 mg $NiCl_2 \cdot 6H_2O$, 36 mg $Na_2MoO_4 \cdot 2H_2O$, 15 mg $Na_2WO_4 \cdot 2H_2O$, 15 mg $Na_2SeO_4 \cdot 5H_2O$.

For sterilization the medium was autoclaved for 20 minutes at 121 °C.

2.2 Reactor set-up

For the fermentations a continuously stirred tank reactor (Biostat BPlus, Sartorius, Germany) with a working volume of 1.5 L was applied. The temperature of the fermentation broth was kept at 70°C, the pH value was maintained at 6.5 using NaOH (1 mol/L) and the stirring rate

was set to 250 rpm. For continuous culture a hydraulic retention time of 15 h was adjusted. The flow rate of nitrogen as stripping gas was adjusted by a Flow Controller Bronkhorst HiTec 1000 mL/min.

Gas production rate was measured continuously by a Ritter gas counter Typ MGC-1 1000 mL/h for the bubble induction fermentation and a Ritter gas counter Typ TG01/5 10 L/h for the underpressure fermentation. For the experiments the tank reactor was sterilized, filled with 1.3 L sterile medium and sparged for 0.5 h with 15 L/h nitrogen. The inoculation was carried out with 250 mL of a *Caldicellulosiruptor saccharolyticus* culture grown overnight at 65 °C.

2.3 Bubble induction experiment

During the continuous operation of the fermentation the inert gas stripping rate was varied, it was stepwise reduced from 5, 3, 1 to 0 L/(h·L). At the different levels stable operation was awaited (ca. 5 retention times). After 75 h and 797 h of continuous operation bubble inducing particles (zeolite, 1 – 2.5 mm, IPUS, Rottenmann, Austria) were added to the fermentation broth. Prior to addition the particles were washed, dried, autoclaved and flushed with nitrogen. In a first step an amount of 10 g/L was added to the fermentation broth, which was increased to a total of 18 g zeolite per liter suspension in a second step. The cysteine amount in the medium was reduced to 0.1 g/L (333 h) and after 510 h of continuous operation, cysteine was omitted completely from the medium.

2.4 Underpressure experiment

For the variation of the underpressure a vacuum diaphragm pump KNF-Vacobox Typ PJ 9988-740.3 was used. The vacuum pump was connected to the head of the fermentor. In a first period the fermentation was performed under normal pressure (1000 mbar) and nitrogen gas stripping of 5 L/(h·L). Subsequently an underpressure of 600 mbar was applied. The nitrogen sparging was abated to 3 L/(h·L). Thereafter nitrogen gas stripping was further reduced to 1 L/(h·L). The underpressure was changed to 400 mbar and afterwards the inert gas stripping omitted completely. The underpressure was finally reduced to 305 mbar before it was raised to 500 mbar. The fermentation was stopped after 20 days of operation.

2.5 Analytical methods

The hydrogen content was determined with a gas chromatograph (PerkinElmer Clarus 500) equipped with a TCD (thermal conductivity detector), a molecular sieve column (Restek RT-Msieve 5A) and a poropak column (Restek Q-Plot). Nitrogen was used as carrier gas with a flow rate of 6 mL/min. The contents of CH4, N2, O2 and CO2 were measured with a gas chromatograph (PerkinElmer Autosystem XL) equipped with a TCD, a molecular sieve column (Restek RT-Msieve 5A) and a poropak column (Restek Q-Plot). As carrier gas helium was used with a flow rate of 6 mL/min. H2S was determined occasionally with a gas chromatograph (PerkinElmer Autosystem XL) equipped with a Restek Rt-XL Sulfur column and a flame photometric detector.

Glucose, acetate and lactate were analysed by a high performance liquid chromatograph (Waters Acquity UPLC) with a refractive index detector and a 300 mm 7.8 mm Aminex HPX-87H column. The optical density of the fermentation broth was determined via absorption at

620 nm using a PerkinElmer Lambda 35 UV/VIS spectrometer. The relation between optical density and cell dry weight was calculated as follows: CDW [g/L] = (0.3796 · OD620nm + 0.0288) (R2 = 0.9984). The measurement of dry matter and organic dry matter was carried out following DIN 38409-H1.

3 Results

3.1 Bubble induction experiment

Figure 1 shows the results of the analysis of the produced gas during the fermentation with induced bubble formation.

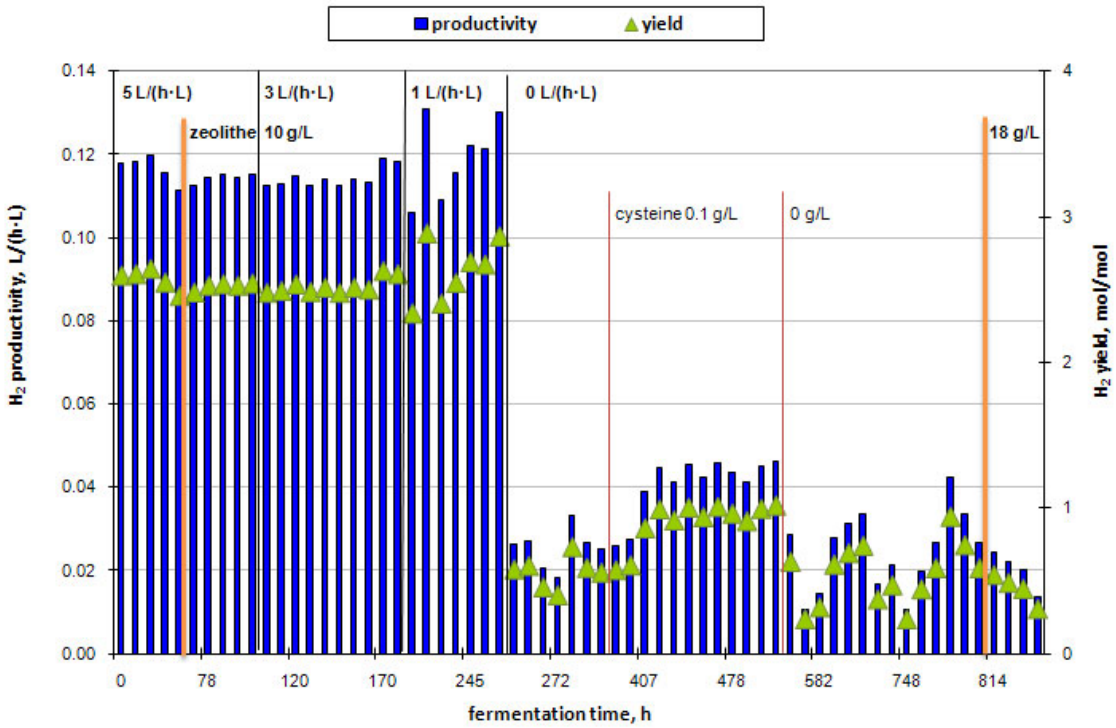

Figure 1: Hydrogen productivity and yield during the bubble induction experiment (*C. saccharolyticus* continuous culture, 15 h HRT).

The hydrogen productivity was constantly above 0.11 L/(h·L) during the first 200 h of the fermentation at a yield of 2.5 mol/mol glucose. The addition of the bubble inductor did not show discernible effects on the production of hydrogen. Similarly the reduction of inert gas stripping to 1 L/(h·l) did not influence the performance of the fermentation to a remarkable extent. Omitting stripping gas completely led to significant lower productivity and yield of 0.025 L/(h·L) and 0.56 mol/mol. The lower cysteine content (after 330 h) improved the production with a certain time delay. With no cysteine in the medium the mean values dropped and showed no continuity. After 800 h of continuous operation a steady decrease in hydrogen production occurred.

During fermentation hydrogen sulphide was found in the produced biogas. With the initial concentration of 0.6 g/L cysteine in the medium nearly 1500 ppm H_2S were detected. The reduced level of 0.1 g/L led to values of 600 ppm. With no cysteine in the medium no H_2S was produced at all.

A part of the inserted zeolite got clamped between the reactor wall and the baffle plate inside of the reactor. There the formation or rather the accumulation of gas bubbles was observed, even after stoppage of nitrogen gas stripping.

After 622 h of continuous operation a short-term switch to batch mode was necessary. The redox potential of the fermentation moved between -450 and -600 mV. The production rates of acids were situated between 2.3 and 14.8 mmol/h. During the experiment some technical problems concerning the pumps for the medium and the effluent occurred. Therefore, the pumps were adjusted manually.

The introduced glucose was totally consumed from the bacteria throughout the continuous operation (data not shown). The maximum concentration of acetic acid was found at the beginning of the fermentation with 2.5 g/L, thereafter the content decreased to 1 g/L. In contrast lactate increased after the strict reduction of gas stripping, after the omission of cysteine from the medium and after the second addition of zeolite. The optical density ranged from 0.45 to 1.05, corresponding to a dry cell weight of 200 to 430 mg/L. After addition of zeolite the optical density reached 1. The reduction of gas stripping to 1 L/(h·L) led to a drop in biomass formation. VFA analysis showed that the only organic acids produced were L-lactic acid and acetic acid.

3.2 Underpressure experiment

Figure 2 shows the results of the analysis of the produced gas during the fermentation with underpressure as method for hydrogen removal.

After the first period of the fermentation the hydrogen values dropped enormously. Changing the position of the vacuum controller led to an increase in hydrogen production. With each reduction of stripping gas the productivity as well as yield decreased. The best performance was achieved at the lowest pressure level of 305 mbar and no nitrogen stripping (0.104 L/(h·L), 2.31 mol/mol). Occasionally, analysis of the produced gas concerning N_2, O_2 and CO_2 concentrations were done. At ambient pressure and nitrogen stripping the gas produced was composed of 90% N_2, below 5% O_2 and CO_2 and about 2% H_2. At a pressure level of 400 mbar and omitted inertgas stripping the following composition was detected: 78% N_2, 20% O_2, 0.7% CO_2 and 1.5% H_2, indicating that the system was not completely tight and air was could enter the system.

The redox potential during fermentation fluctuated between -350 and -470 mV. The latter value was found at a pressure of 305 mbar without nitrogen stripping. The consumption rate of sodium hydroxide solution stayed constant throughout the fermentation. 3.6 mmol/h of organic acids were produced.

Glucose (data not shown) was entirely consumed by the bacteria until the end of the experiment, the concentration of unused sugar increased to more than 1 g/L. The acetate concentration fluctuated between 2 and 2.5 g/L. The metabolite lactate could not be detected throughout the fermentation. The lowest optical density was 0.7 which corresponded to a cell

dry weight of 300 mg/L. The highest biomass content was reached at a pressure of 305 mbar (OD 1.2, CDW 500 mg/L).

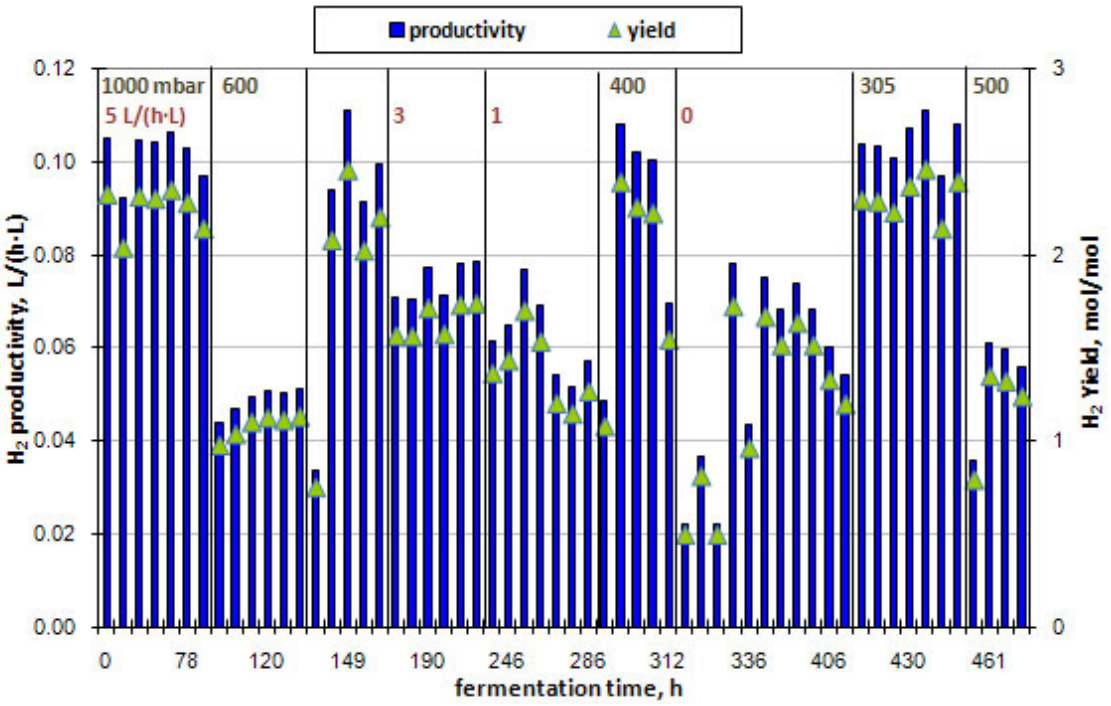

Figure 2: Hydrogen productivity and yield during the underpressure experiment (C. saccharolyticus continuous culture, 15 h HRT).

4 Discussion and Conclusion

The addition of a zeolite to induce bubble formation showed no direct effects on the fermentation. However a reduction of stripping gas to 1 L/(h·L) was possible without influencing the performance of hydrogen production. Hydrogen productivities of 0.115 L/(h·L) and yields of 2.5 mol/mol were achieved. In contrast to reference fermentations, inert gas stripping on a very low level was possible due to the bubble formation without reduced hydrogen productivity and yield. Omitting nitrogen sparging led to a radical decrease in hydrogen production, indicating that the produced hydrogen was not removed sufficiently from the liquid phase and led to an end product inhibition. By the use of zeolite the fermentation could be maintained without gas stripping but with far lower hydrogen yields and productivities. Therefore it is not profitable to ferment without nitrogen gas stripping to achieve adequate hydrogen values. Remarkably, no effect of the quantity of added zeolite could be detected, which one could expect. In contrast the bacterial growth was declining, when increasing the amount of zeolite. Anyway addition of zeolite caused an increase of the optical density, which could be due to the abrasion of the material. The gradual reduction of cysteine in the medium was necessary due to the high hydrogen sulphide content in order to avoid inhibition of the microorganisms and consumption of hydrogen for H_2S formation. The reduction of cysteine showed a positive effect on the fermentation. However, without cysteine

the fermentation became more unstable. The determination of VFAs showed that only lactic and acetic acid were produced. The carbon balance was considerably below 100 % indicating that other metabolites must have been produced. However, the results showed that the addition of bubble inducing materials was beneficial to a certain extent, but could not replace insertion of stripping gas to 100%. These findings could explain the better performance of fermentor systems using carrier materials due to enhanced bubble formation on the one hand and due to increased retention of biomass on the other [5, 6].

During each section of fermentation with underpressure the hydrogen productivities and yields remained almost constant. The appliance of underpressure led at first to a bisection of hydrogen production, which normalized after repositioning of the vacuum controller, avoiding high pressure surges and keeping up a stable vacuum. With each reduction of the stripping gas flow a decline of hydrogen values was observed, whereas each decrease of pressure led to an increased hydrogen production. The best data were obtained at a pressure level of 305 mbar without nitrogen sparging (0.100 L/(h·L) and 2.3 mol/mol hexose). Similar data were only achieved when applying high inert gas stripping rates (5 L/(h·L)) and pressures of 1000 or 600 mbar. Therefore underpressure is an applicable alternative to inert gas stripping for reduction of hydrogen partial pressure.

At the end of the fermentation around 20 % of the provided sugar was not consumed. This indicated, like the decrease in biomass, the death or rather the reduced metabolism of the bacteria. Therefore an applied underpressure of 500 mbar was not sufficient for stable operation of the fermentation. The progress of the experiment with successive increase of applied vacuum indicated that a further increase might lead to higher hydrogen productivities. This thesis was not tested because pressures lower than 300 mbar induced boiling of the fermentation broth at the used temperature of 70 °C.

Another approach for optimization is the reduction of the hydraulic retention time and hence higher organic loads. For further experiments it is certainly of great importance to get the whole system as vacuum-sealed as possible to maintain the underpressure in the tank reactor. Anyway the vacuum pump was not aligned to this small scale experiment and turned out to be too powerful. However, for the large-scale application vacuum-sealed systems are state of the art and are already in use for the production of bioethanol [7, 8, 9].

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