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# Heading for an economic industrial upgrading of crude glycerol from biodiesel production to 1,3-propanediol by *Lactobacillus diolivorans*

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## HIGHLIGHTS

- *Lactobacillus diolivorans* efficiently converts crude glycerol to 1,3-propanediol.
- *L. diolivorans* is not inhibited by 0.7 g/l furfural and 0.3 g/l 5-HMF.
- Conversion of biobased resources to up to 85 g/l with a productivity of 0.45 g/l h.

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## ABSTRACT

*Lactobacillus diolivorans* was evaluated as a potential organism for production of 1,3-propanediol under industrially relevant conditions. Crude glycerol of different origins has been tested and showed no inhibitory effects on growth or production. Using crude glycerol from biodiesel production from palm oil 85 g/l 1,3-propanediol have been obtained with a productivity of 0.45 g/l h in a fed-batch cultivation. Sugar necessary for the formation of biomass was replaced with a hydrolysate from lignocellulosic material resulting in 75 g/l 1,3-propanediol and a productivity of 0.36 g/l h. Lignocellulosic hydrolysate contained the potential inhibitors furfural and 5-hydroxymethylfurfural at concentrations of 0.7 and 0.3 g/l, respectively. Addition of furfural and 5-hydroxymethylfurfural to batch cultures in said concentrations did not show inhibitory effects on growth or 1,3-propanediol production.



## 1. Introduction

The production of biodiesel increased dramatically over the past years (Almeida et al., 2012). Biodiesel is produced via transesterification. Plant fat or oil reacts with an alcohol (usually methanol) to fatty acid (FA) esters, thereby liberating glycerol (Ma and Hanna, 1999). Glycerol from biodiesel production, referred to as crude or raw glycerol, amounts to 10% of the total biodiesel production volume (Almeida et al., 2012). The overall economic efficiency of biodiesel production depends on glycerol as additional source of income. However, with increasing production of biodiesel, glycerol prices have seen a sharp decrease with prices as low as $110/t (Kerr et al., 2007). Therefore, glycerol has become a waste product rather than a by-product of biodiesel production (Yang et al., 2012). In order to improve income of a biodiesel biorefinery, glycerol has to undergo a value-adding step to produce high-value chemicals such as 1,3-propanediol.

However, crude glycerol from biodiesel production contains a number of impurities such as methanol (usually used for transesterification), triglycerides, salts (as catalyst), moisture and soap (Yang et al., 2012). Some of the impurities, in particular free fatty acids, have been reported to be inhibiting for microbial fermentations such as the production of 1,3-propanediol production with *Clostridium butyricum* (Petitdemange et al., 1995; Chatzifragkou et al., 2010; Chatzifragkou and Papanikolaou, 2012).

As reported previously, *Lactobacillus diolivorans* is a good natural producer of 1,3-propanediol from glycerol (Pflügl et al., 2012). However, 1,3-propanediol production with *L. diolivorans* requires the addition of a sugar (e.g. glucose), as the organism is not able to grow on glycerol as the main source of carbon. Pure D-glucose is an expensive carbon source for biomass formation, thereby increasing production costs. Hydrolysates from lignocellulosic material are a cheap alternative (Heer and Sauer, 2008). Lignocellulosic hydrolysates often contain toxic compounds which may inhibit microbial fermentations, as reported for example for production of ethanol with *Saccharomyces cerevisiae* (Palmqvist and Hahn-Hägerdal, 2000; Almeida et al., 2007).

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The overarching aim of this study was to evaluate the potential of *L. diolivorans* as a production host for 1,3-propanediol under actual industrial and economic conditions. Therefore, the ability of *L. diolivorans* to produce 1,3-propanediol with crude glycerol from different origins was tested. D-glucose was replaced by lignocellulosic hydrolysate and potential inhibitory effects have been evaluated.

## 2. Methods

### 2.1. Microorganism and medium

*L. diolivorans* DSM 14421 (LMG 19667) was used for all experiments in this study. Cells were maintained at −80 °C in culture broth supplemented with 10% (w/v) glycerol.

MRS medium as developed by De Man et al. (1960) was used in a modified form for all cultivations in this study (Pflügl et al., 2012). For the batch phase, MRS was supplemented with 3% (w/v) D-glucose or other sugars and 1% (w/v) pharma grade or crude glycerol.

During fed-batch cultivations a glucose/glycerol solution with a molar ratio of 0.1 was used as feed medium. The concentration of glycerol in the feed solution was 500 g/l and the concentration of D-glucose was 97.8 g/l. For cultivations with lignocellulosic hydrolysate the total sugar content was adjusted to 97.8 g/l. The actual concentrations of the feed solution were determined for all cultivations and used for the calculations and showed deviations of no more than 20%. 5 mg/l vitamin $B_{12}$ was added to the batch and feed medium in all fed-batch cultivations. 100% (w/v) Struktol® SB 2121 (Schill + Seilacher, Hamburg, Germany) was used as antifoam agent for the fed-batch cultures and was only added as necessary.

### 2.2. Preparation of crude glycerol

Crude glycerol was obtained as follows: the crude suspension from the biodiesel production process containing glycerol was adjusted to pH 7, when necessary, and autoclaved for 20 min at 121 °C. A two phase system was obtained, with an organic phase containing fatty acids and an aqueous phase containing glycerol. Subsequently, the organic phase was removed. The glycerol concentration from the aqueous phase was determined by HPLC analysis and used as a stock solution for addition to the culture medium.

### 2.3. Batch and fed-batch cultures

For all cultivations the fedbatch-pro® bioreactor system (DASGIP AG, Jülich, Germany) was used. Technical specifications of the reactor system, preparation of cultivations and culture conditions were as described previously (Pflügl et al., 2012).

For all cultivations, 700 ml culture medium was inoculated to an $OD_{600}$ of 0.1 with 2% (v/v) inoculum from an exponentially growing preculture. For the fed-batch cultivations, the separately sterilized feed solution was added at a rate of 1.5 ml/h after glycerol was consumed from the batch medium.

### 2.4. Analytical procedures

12 ml samples were taken at regular intervals throughout the whole cultivation duration. Biomass production was determined by measuring optical density at 600 nm. $OD_{600}$ values were converted into cell dry mass (CDM) with a previously established correlation (Pflügl et al., 2012). The lowest CDM concentration detectable is 0.125 g/l derived from the lowest detectable $OD_{600}$ value of 0.1.

The concentrations of D-glucose, D-xylose, D-fructose, L-arabinose, glycerol, 1,3-propanediol, 3-hydroxypropionic acid, lactic acid, acetic acid and ethanol in the culture broth were determined by HPLC analysis (Shimadzu, Korneuburg, Austria) with a Rezex ROA-Organic Acid H + column (300 mm × 7.8 mm, Phenomenex, USA). The column was operated at 60 °C, 1.0 ml/min flow rate and 0.004 M $H_2SO_4$ as mobile phase. Detectors used were a refraction index detector (RID-10A, Shimadzu, Korneuburg, Austria) and a photodiode array detector (SPD-M20A, Shimadzu, Korneuburg, Austria). Sample preparation and detection limits of HPLC measurements were as described previously (Pflügl et al., 2012).

Lignocellulosic hydrolysate was analyzed with a Rezex RPM Monosaccharide Pb + 2 column (300 mm × 7.8 mm, Phenomenex, USA). The column was operated at 80 °C temperature, 0.6 ml/min flow rate and water as mobile phase (same detectors as above). Samples were filtrated, and 10 µl of sample were injected for analysis of D-glucose, D-xylose, L-arabinose, mannose, galactose, furfural and 5-hydroxymethyl furfural. Technical replicates of the analyses were within a 5% margin.

$CO_2$ in the fermentation off-gas was quantified. Together with biomass concentrations and the produced metabolites carbon balances were set up as described previously (Pflügl et al., 2012). Within the margin of error, complete carbon recovery was observed for all cultivations.

### 2.5. Preparation of lignocellulosic hydrolysate

Commercially available wood chips for heating purposes containing 30% (w/w) spruce and 70% (w/w) beech were treated with steam explosion for 15 min at 121 °C and 2 bar pressure. Samples were stored at −20 °C until they were further treated. For enzymatic digestion, dry matter of the sample was determined, which ranged between 20% and 30% (w/w). The amount of dry material was adjusted to 20 g/l with water, and Cellic® enzyme (Novozymes®, USA) according to the manufactures recommendation. Digestions were performed in 2 l shake flasks at 50 °C and 220 rpm. After 72 h, digestion was completed. The final solution of lignocellulosic hydrolysate was obtained by centrifugation and used as a stock for preparation of either batch or feed medium.

Lignocellulosic hydrolysates contained up to 58 and 18 g/l D-glucose and D-xylose, respectively. The final feed medium contained 97.8 g/l total sugars and 500 g/l glycerol. Pure D-glucose and D-xylose were added in the same ratio as contained in the original lignocellulosic hydrolysate when necessary.

## 3. Results and discussion

### 3.1. Different crude glycerols in fed-batch with L. diolivorans DSM 14421

As reported previously, *L. diolivorans* is a good producer of 1,3-propanediol from glycerol in a fed-batch process cofermenting D-glucose and glycerol (Pflügl et al., 2012). For an industrial scale production process, glycerol would not be used as pharma grade glycerol, but as crude glycerol produced during biodiesel production. However, this form of glycerol contains a number of impurities, some of which have been reported to have inhibitory effects on microbial fermentations. The source of inhibition has been identified mainly as free fatty acids remaining after incomplete transesterification (Venkataramanan et al., 2012; Nguyen et al., 2013). Subsequently, the production potential of 1,3-propanediol by *L. diolivorans* with crude glycerol was evaluated in fed-batch cultivations. The cultivations were carried out on MRS medium supplemented with 3% (w/v) D-glucose, 1% (w/v) crude glycerol as batch medium and a glucose-glycerol solution as feed. Glucose is

required for biomass formation during the batch phase, and during the feed phase as supplier of energy (via ATP) and reduction equivalents (via NADH) for 1,3-propanediol production. The crude glycerol used in this study was the by-product from industrial biodiesel plants using either canola (Rossi Biofuel Zrt., Komáron, Hungary) or palm oil (Thai Oleochemical Co. Ltd., Thailand) for production of biodiesel.

Fig. 1A–C shows the results of fed-batch cultivations with crude glycerols from palm oil and canola compared to a fed-batch with pharma grade glycerol. 1,3-Propanediol concentrations obtained from palm oil and canola crude glycerol cultivations were in the same range compared to the pharma grade glycerol cultivation (85, 75 and 84 g/l, respectively). Productivity parameters as well as product yields for total and glycerol carbon were comparable to a cultivation with D-glucose and pharma grade glycerol (Table 1). Biomass formation was comparable for all three cultivations. Also the concentration of acetic acid formed during crude glycerol cultivations was in the same range as for the pharma grade glycerol cultivation. Lactic acid and ethanol mainly accumulated during the batch phase, and were subsequently consumed during the feed phase of all three cultivations, as reported previously (Da Cunha and Foster, 1992; Pflügl et al., 2012). Additionally, some 3-hydroxypropionic acid accumulated. The results of these cultivations do not show any inhibitory effects of crude glycerol from canola and palm oil on growth or formation of 1,3-propanediol by *L. diolivorans*. Furthermore, palm oil-based crude glycerols from other sources were tested, leading to comparable 1,3-propanediol concentrations without any inhibitory effect. This clearly indicates that crude glycerol from different sources can be used for efficient 1,3-propanediol production with *L. diolivorans*.

To test *L. diolivorans* as a promising organism for industrial production of 1,3-propanediol, the described fed-batch process using crude glycerol originating from palm oil was up-scaled to a volume of 50 l. Cultivations carried out in 50 l scale showed 1,3-propanediol concentrations of up to 83 g/l and volumetric productivities of up to 0.45 g/l h, comparable to small scale cultivations (Fig. 1D and Table 1). These results show that the fed-batch process can be scaled up to 50 l scale without decreasing 1,3-propanediol concentration, implying that the process at hand is very robust and possibly suitable for industrial production of 1,3-propanediol with *L. diolivorans*. However, one point to be considered is the requirement of vitamin $B_{12}$ of *L. diolivorans* to obtain the 1,3-propanediol concentrations reported in this study. Generally, exogenous vitamin $B_{12}$ represents a cost factor that renders a process potentially less economical (Adkins et al., 2012). In case of this *L. diolivorans* based process, addition of 5 mg/l vitamin $B_{12}$ is sufficient for optimal performance. Considering the range of prices at which vitamin $B_{12}$ is offered at the world market, and the value obtainable for 1,3-propanediol this amount appears not as a major shortcoming of this fed-batch process. Nevertheless, strain improvement by

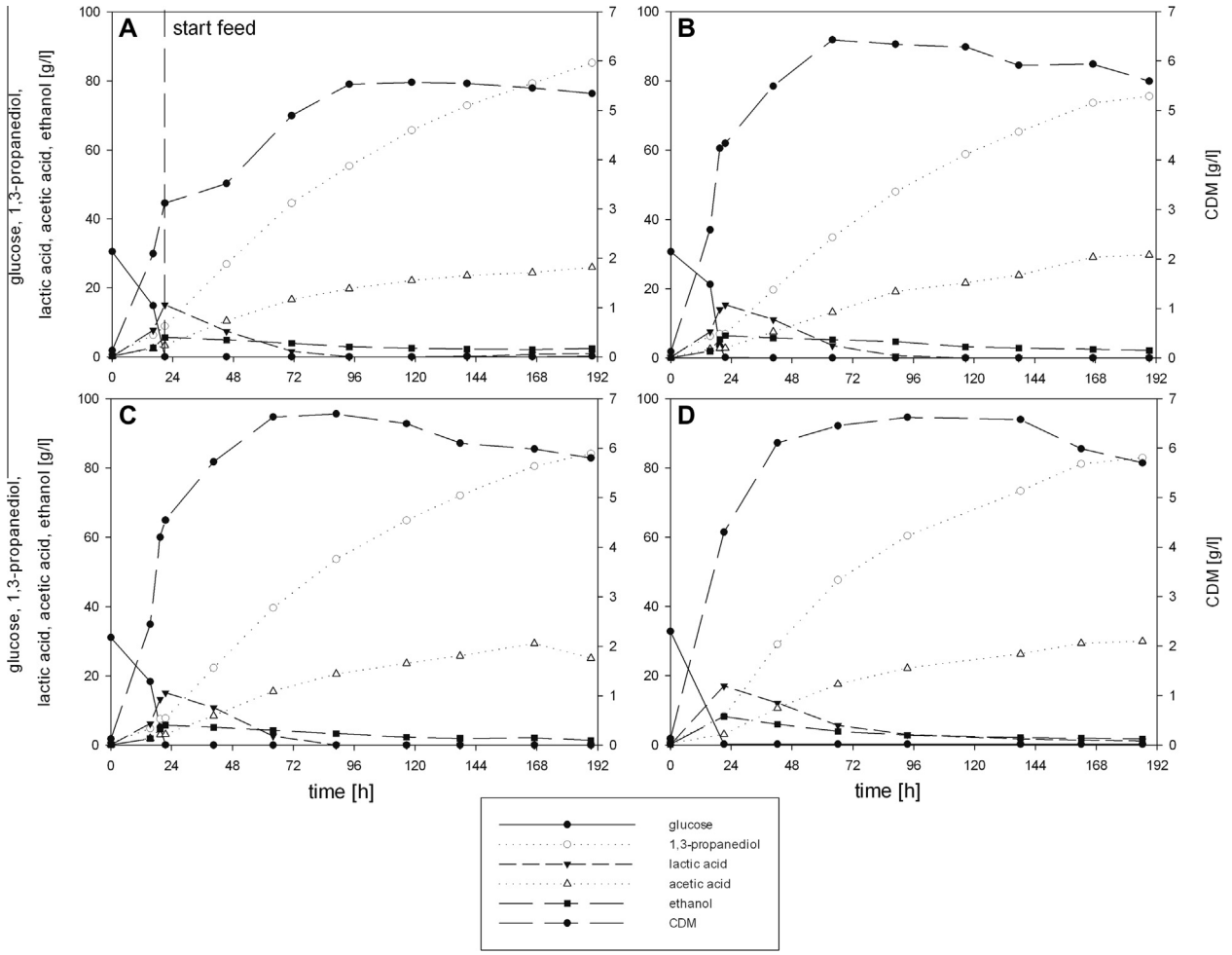


**Fig. 1.** Time course of fed-batch cultivations of *L. diolivorans* with crude glycerol originating from biodiesel production with (A) palm oil and (B) canola. (C) Fed-batch cultivation with pharma grade glycerol. (D) 50 l fed-batch cultivation with crude glycerol from palm oil. Experiments A–C were done at least in duplicate, experiment D was done five times. One experiment is shown as an example. Deviations of repetitions never exceeded 5%.

**Table 1**
Overview of $Q_p$, $q_p$ and $Y_{P/S}$ for fed-batch cultivations of *L. diolivorans* cofermenting either crude glycerol (palm oil and canola) and D-glucose, crude glycerol and lignocellulosic hydrolysate or pharma grade glycerol and D-glucose as comparison. The values for $Q_p$ and $q_p$ represent productivities during the feed phase and were determined as described previously (Pflügl et al., 2012). Since two or more substrates were used during the cultivations, product yields for total carbon and glycerol carbon were calculated using C-mol for each substance. The values given in the table represent the mean value of two individual experiments.

| Parameter | Crude glycerol (palm oil) + glucose | Crude glycerol (palm oil) + glucose (50 l scale) | Crude glycerol (canola) + glucose | Crude glycerol (palm oil) + lignocellulosic hydrolysate | Pharma grade glycerol + glucose |
|---|---|---|---|---|---|
| $Q_{p\ \text{average feed}}$ [$g_{1,3\text{-propanediol}}$/l h] | 0.45 | 0.45 | 0.41 | 0.36 | 0.46 |
| $q_{p\ \text{average feed}}$ [$g_{1,3\text{-propanediol}}/g_{CDM}$ h] | 0.10 | 0.08 | 0.07 | 0.06 | 0.08 |
| $Y_{P/S}$ [$mol_{C\ 1,3\text{-propanediol}}/mol_{C\ \text{substrate total}}$] | 0.49 | 0.47 | 0.44 | 0.54 | 0.47 |
| $Y_{P/S}$ [$mol_{C\ 1,3\text{-propanediol}}/mol_{C\ \text{glycerol}}$] | 0.66 | 0.64 | 0.61 | 0.76 | 0.62 |

genetic engineering might make the addition of vitamin $B_{12}$ obsolete in the future (Pflügl et al. 2013).

### 3.2. Batch cultivations with different sugars and glycerol

In order to establish an economic industrial production process for 1,3-propanediol, cost efficient production with low cost substrates is required. D-glucose has been shown to be required for biomass formation in the production process of 1,3-propanediol from glycerol with *L. diolivorans*. However, pure D-glucose is an expensive carbon source. In order to lower production costs, the ability of *L. diolivorans* to utilize pentoses such as D-xylose and L-arabinose was evaluated. These sugars, together with D-glucose are the main sugars found in hydrolysates prepared from lignocellulosic material.

D-Fructose, D-xylose and L-arabinose were tested regarding biomass formation as well as 1,3-propanediol production. Two series of cultivations were carried out on MRS medium supplemented with 2% (w/v) of the respective sugar. To one series 1% (w/v) glycerol was added, the other one was cultivated without glycerol.

Biomass formation for cultivations without glycerol was 10% and 45% lower for L-arabinose and D-fructose, respectively, whereas biomass formation with D-xylose was 60% higher compared to D-glucose (Table 2A). The main fermentation products of L-arabinose and D-xylose were lactic acid and acetic acid and only a small amount of ethanol, whereas for D-glucose formation of lactic acid, acetic acid and ethanol was observed. The main fermentation product for D-fructose was mannitol with smaller amounts of lactic acid and acetic acid (Table 2A). When glycerol was added to the culture medium biomass formation was 7%, 10%, and 20% lower for D-fructose, D-xylose and L-arabinose, respectively, compared to D-glucose (Table 2B). 1,3-Propanediol formation for cofermentations with glycerol was observed for D-fructose and D-xylose, where 120% and 40% of the 1,3-propanediol concentrations obtained for glucose-glycerol cultivation was observed (Table 2B). No 1,3-propanediol formation was observed for L-arabinose-glycerol cultivation. The lower concentration of 1,3-propanediol obtained for D-xylose can be explained by the lower amount of NADH produced from D-xylose compared to D-glucose (1 mol instead of 2 mol NADH per mol sugar). However, D-xylose and L-arabinose are catabolized via the same metabolic pathway. Therefore, it is somewhat surprising that no 1,3-propanediol production was observed when L-arabinose and glycerol were co-fermented. One explanation might be a difference in the enzyme kinetics of the first steps of D-xylose and L-arabinose utilization, upstream of the common intermediate xylulose 5-phosphate. Assuming a faster utilization of L-arabinose compared to D-xylose, this would lead to a higher amount of pyruvate, which could be converted to a higher extent to lactic acid, therefore decreasing the amount of NADH available for 1,3-propanediol production from glycerol. In conclusion, cofermentation of different pentoses and hexoses with glycerol for 1,3-propanediol production could be shown.

### 3.3. Fed-batch cultivations with crude glycerol and lignocellulosic hydrolysate from wood chips

The use of lignocellulosic hydrolysates as potential replacement of pure sugars for biomass formation and 1,3-propanediol production with *L. diolivorans* has been evaluated. Utilization of crude glycerol did not show any inhibiting effects on 1,3-propanediol production. Fed-batch cultivations were carried out using the lignocellulosic hydrolysate as carbon source for biomass formation in the batch medium, or during the feed phase as supplier of energy (via ATP) and reduction equivalents (via NADH) for 1,3-propanediol production. Additionally, a mixture of pure glucose and xylose in the same concentrations and ratio as in the lignocellulosic hydrolysate was used in order to test for any inhibitory effects from the lignocellulosic hydrolysate.

The two cultivations with lignocellulosic hydrolysate and the glucose/xylose mixture in the batch medium as well as the cultivation with lignocellulosic hydrolysate in the feed medium all showed comparable 1,3-propanediol concentrations (80.0, 77.5

**Table 2**
Overview of the biomass and metabolites determined at the end of the batch cultivations (72 h) of *L. diolivorans* on MRS with (A) 2% (w/v) sugar and (B) 2% (w/v) sugar and 1% (w/v) glycerol. The values given in the table represent the mean value of six individual experiments.

| (A) Sugar | CDM [g/l] | Sugar [g/l] | Mannitol [g/l] | Lactic acid [g/l] | Acetic acid [g/l] | Ethanol [g/l] |
|---|---|---|---|---|---|---|
| L-Arabinose | 2.4 | 0.5 | – | 10.0 | 11.8 | 0.3 |
| D-Fructose | 1.6 | 0.0 | 13.8 | 3.4 | 6.4 | 0.2 |
| D-Glucose | 2.8 | 0.1 | – | 9.7 | 4.5 | 5.7 |
| D-Xylose | 4.3 | 0.0 | – | 7.8 | 12.9 | 0.4 |

| (B) Sugar | CDM [g/l] | Sugar [g/l] | Mannitol [g/l] | Lactic acid [g/l] | Acetic acid [g/l] | Ethanol [g/l] | Glycerol [g/l] | 1,3-Propanediol [g/l] |
|---|---|---|---|---|---|---|---|---|
| L-Arabinose | 2.5 | 0.4 | – | 9.8 | 11.4 | 0.5 | 9.8 | 0.1 |
| D-Fructose | 2.8 | 0.0 | 4.5 | 5.8 | 9.0 | 0.3 | 0.0 | 8.4 |
| D-Glucose | 3.0 | 0.0 | – | 9.2 | 6.7 | 3.9 | 1.3 | 7.0 |
| D-Xylose | 2.7 | 0.2 | – | 8.8 | 12.5 | 0.7 | 5.4 | 2.9 |

and 78.7 g/l, respectively) (Fig. 2A–C). The major part of the glycerol fed to the cultures was converted into 1,3-propanediol. As for the cultivations with crude glycerol, accumulation of 3-hydroxypropionic acid was observed. However, biomass and 1,3-propanediol formation during the batch phase of the two cultivations with lignocellulosic and the sugar solution in the batch medium was slower compared to the cultivation with pure D-glucose in the batch (~5 h). At the end of the batch, however, the same biomass and 1,3-propanediol concentration were obtained.

Conceivable causes of the prolonged batch phase include (i) inhibition by potentially toxic compounds contained in the lignocellulosic hydrolysate and (ii) slower utilization of D-xylose compared to D-glucose.

In fact, the prolonged batch phase appears to be caused by slower utilization of D-xylose. This is supported by the fact that the prolonged batch phase was observed for the cultivation on lignocellulosic hydrolysate as well as for a culture where D-glucose was substituted by a mixture of D-glucose and D-xylose. Although both sugars are to some extent catabolized simultaneously, at the time of total glucose consumption 3.2 g/l D-xylose were left in the medium (Fig. 2A and B).

The potentially toxic and inhibitory substances of lignocellulosic hydrolysates are formed during pretreatment of biomass for example via steam explosion. Main inhibitors have been identified as phenolic compounds (e.g. ferulic acid), weak acids (e.g. acetic acid) and furfurals (e.g. furfural and 5-hydroxymethyl furfural) (Mills et al., 2009). There are numerous reports mainly for yeast and ethanol fermentation reporting reduced biomass and ethanol formation caused by inhibition from lignocellulosic hydrolysate (Palmqvist and Hahn-Hägerdal, 2000; Almeida et al., 2007). However, several members of the genus *Lactobacillus* have been shown to be quite resistant to inhibition by substances usually contained in lignocellulosic hydrolysate (Guo et al., 2010; van Niel et al., 2012). For example, it is reported that *Lactobacillus reuteri* converts furfural and 5-hydroxymethyl furfural into their respective alcohols, thereby using them as electron acceptors (van Niel et al., 2012). The lignocellulosic hydrolysate used for the fed-batch cultivations in this study contained 0.7 and 0.3 g/l furfural and 5-hydroxymethyl furfural (5-HMF), respectively. Addition of furfural or 5-HMF or both to batch cultures of *L. diolivorans* at these concentrations did not show any inhibiting effects on growth or production of 1,3-propanediol (data not shown). Therefore, inhibition can be ruled out as potential explanation for the prolonged batch phase under the tested conditions.

The cultivation with lignocellulosic hydrolysate in the batch medium showed a higher acetic acid concentration compared to

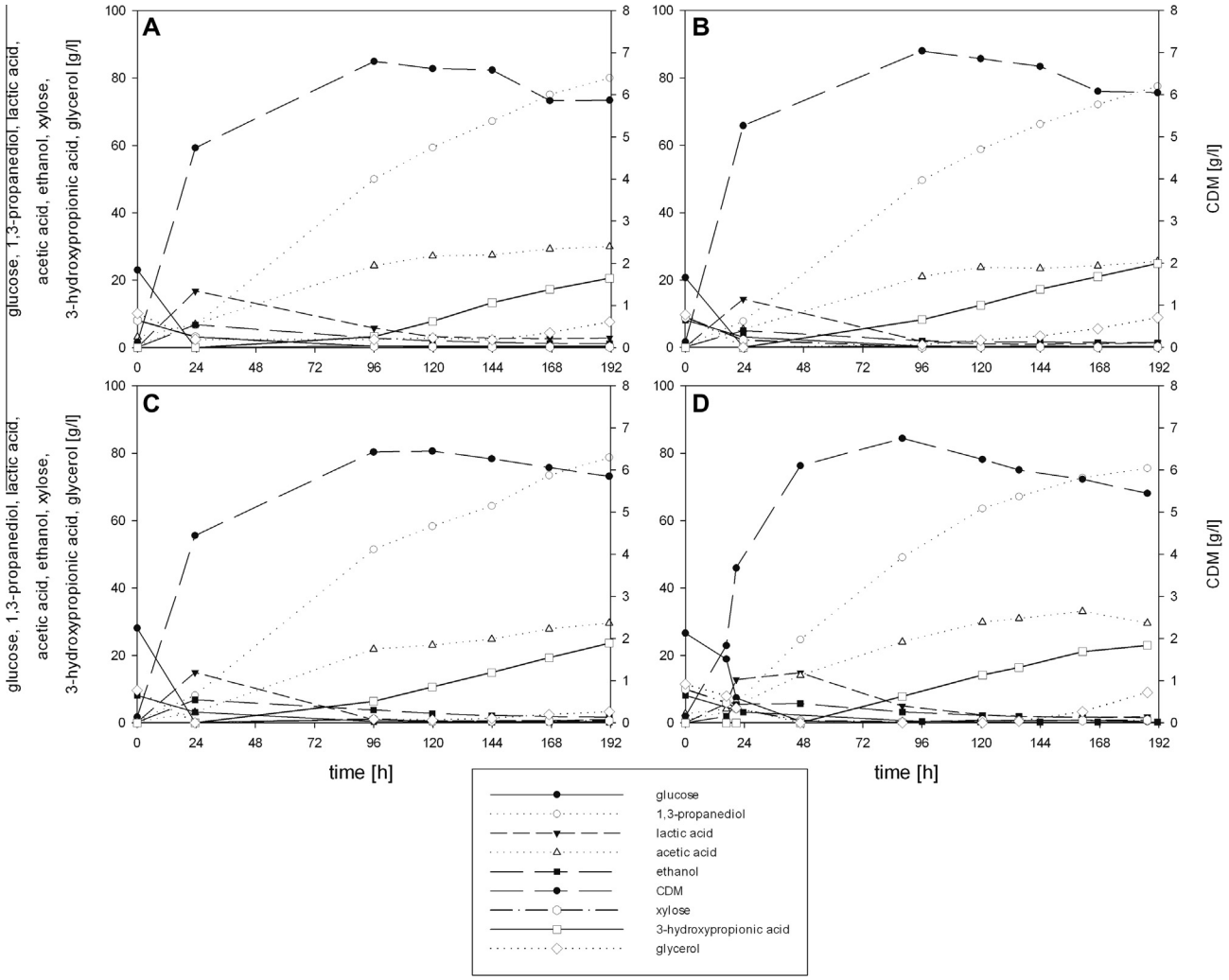


**Fig. 2.** Time course of fed-batch cultivations of *L. diolivorans* with lignocellulosic hydrolysate. (A) Lignocellulosic hydrolysate in the batch medium, (B) mixture of glucose and xylose in the batch medium, (C) lignocellulosic hydrolysate in the feed medium and (D) lignocellulosic hydrolysate in batch and feed medium. All experiments were carried out in duplicate. One experiment is shown as an example. Deviations of repetitions never exceeded 5%.

the cultivation with the mixture of glucose and xylose in the batch medium and the cultivation with lignocellulosic hydrolysate in the feed (30, 25 and 25 g/l, respectively). However, acetic acid could be shown not to be inhibitory for *L. diolivorans* even at concentrations as high as 60 g/l (data not shown).

The use of lignocellulosic hydrolysate for both, the batch and the feed phase was successful. The comparison with the cultivation where a mixture of glucose and xylose was used did not reveal any inhibiting effects. Clearly, the potential concentration of inhibiting substances from the lignocellulosic hydrolysates remains also limited due to the feed strategy. This underlines once more the industrial applicability of the suggested process.

To evaluate the applicability of lignocellulosic hydrolysate as the sole source of sugar during batch and feed phase, a fed-batch cultivation was carried out where crude glycerol from palm oil origin was used for conversion into 1,3-propanediol.

The cultivation shows a final 1,3-propanediol concentration of 75 g/l, which is only slightly lower compared to a cultivation with pure D-glucose in batch and feed medium (Fig. 2D). Also formation of biomass and 3-hydroxypropionic acid is comparable. The acetic acid concentration was slightly increased compared to a cultivation with pure D-glucose, due to the acetic acid contained in lignocellulosic hydrolysate. Furthermore, crude glycerol was mostly converted into 1,3-propanediol and some 3-hydroxypropionic acid. Productivity parameters as well as product yields for total and glycerol carbon were comparable to a cultivation with D-glucose and pharma grade glycerol (Table 1).

The results of this cultivation show the ability of *L. diolivorans* to combine efficient production of 1,3-propanediol from crude glycerol with the use of lignocellulosic hydrolysate as the sole sugar source for biomass formation and production of energy and reduction equivalents.

## 4. Conclusion

*L. diolivorans* can efficiently convert crude glycerol resulting from biodiesel production to 1,3-propanediol. Palm oil derived glycerol and canola derived glycerol are equally well converted. The process has been upscaled to 50 l scale and proved to be very robust. *L. diolivorans* is able to use lignocellulosic hydrolysate as sole sugar source in combination with crude glycerol for efficient production of 1,3-propanediol. It is therefore a very interesting microbial biocatalyst for industrial scale economic production of 1,3-propanediol. Pilot scale tests of the disclosed process will be the next step for the development of an industrial process for 1,3-propanediol manufacture.

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## References

Adkins, J., Pugh, S., McKenna, R., Nielsen, D.R., 2012. Engineering microbial chemical factories to produce renewable "biomonomers". Front. Microbiol. 3, 313.
Almeida, J.R., Modig, T., Petersson, A., Hähn-Hägerdal, B., Lidén, G., Gorwa-Grauslund, M.F., 2007. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J. Chem. Technol. Biotechnol. 82, 340–349.
Almeida, J.R.M., Favaro, L.C.L., Quirino, B.F., 2012. Biodiesel biorefinery: opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnol. Biofuels 5, 48.
Chatzifragkou, A., Papanikolaou, S., 2012. Effect of impurities in biodiesel-derived waste glycerol on the performance and feasibility of biotechnological processes. Appl. Microbiol. Biotechnol. 95, 13–27.
Chatzifragkou, A., Dietz, D., Komaitis, M., Zeng, A.-P., Papanikolaou, S., 2010. Effect of biodiesel-derived waste glycerol impurities on biomass and 1,3-propanediol production of *Clostridium butyricum* VPI 1718. Biotechnol. Bioeng. 107, 76–84.
Da Cunha, M.V., Foster, M.A., 1992. Sugar-glycerol cofermentations in *lactobacilli*: the fate of lactate. J. Bacteriol. 174, 1013–1019.
De Man, J.C., Rogosa, M., Sharpe, M.E., 1960. A Medium for the Cultivation of *Lactobacilli*. J. Appl. Microbiol. 23, 130–135.
Guo, W., Jia, W., Li, Y., Chen, S., 2010. Performances of *Lactobacillus brevis* for producing lactic acid from hydrolysate of lignocellulosics. Appl. Biochem. Biotechnol. 161, 124–136.
Heer, D., Sauer, U., 2008. Identification of furfural as a key toxin in lignocellulosic hydrolysates and evolution of a tolerant yeast strain. Microb. Biotechnol. 1, 497–506.
Kerr, B., Dozier Iii, W., Bregendahl, K., 2007. Nutritional value of crude glycerin for nonruminants. J. Anim. Sci., 6–18.
Ma, F., Hanna, M.A., 1999. Biodiesel production: a review. Bioresour. Technol. 70, 1–15.
Mills, T.Y., Sandoval, N.R., Gill, R.T., 2009. Cellulosic hydrolysate toxicity and tolerance mechanisms in *Escherichia coli*. Biotechnol. Biofuels 2, 26.
Nguyen, A.D.Q., Kim, Y.G., Kim, S.B., Kim, C.-J., 2013. Improved tolerance of recombinant *Escherichia coli* to the toxicity of crude glycerol by overexpressing trehalose biosynthetic genes (otsBA) for the production of β-carotene. Bioresour. Technol. 143, 531–537.
Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresour. Technol. 74, 25–33.
Petitdemange, E., Dürr, C., Abbad Andaloussi, S., Raval, G., 1995. Fermentation of raw glycerol to 1,3-propanediol by new strains of *Clostridium butyricum*. J. Ind. Microbiol. Biotechnol. 15, 498–502.
Pflügl, S., Marx, H., Mattanovich, D., Sauer, M., 2012. 1,3-Propanediol production from glycerol with *Lactobacillus diolivorans*. Bioresour. Technol. 119, 133–140.
Pflügl, S., Marx, H., Mattanovich, D., Sauer, M., 2013. Genetic engineering of *Lactobacillus diolivorans*. FEMS Microbiol. Lett. 344, 152–158.
van Niel, E.W.J., Larsson, C.U., Lohmeier-Vogel, E.M., Rådström, P., 2012. The potential of biodetoxification activity as a probiotic property of *Lactobacillus reuteri*. Int. J. Food Microbiol. 152, 206–210.
Venkataramanan, K.P., Boatman, J.J., Kurniawan, Y., Taconi, K.A., Bothun, G.D., Scholz, C., 2012. Impact of impurities in biodiesel-derived crude glycerol on the fermentation by *Clostridium pasteurianum* ATCC 6013. Appl. Microbiol. Biotechnol. 93, 1325–1335.
Yang, F., Hanna, M.A., Sun, R., 2012. Value-added uses for crude glycerol – a byproduct of biodiesel production. Biotechnol. Biofuels 5, 13.