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BIOENERGY/BIOFUELS/BIOCHEMICALS

Fermentation of crude glycerol from biodiesel production by *Clostridium pasteurianum*

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Abstract *Clostridium pasteurianum* can utilize glycerol as the sole carbon source for the production of butanol and 1,3-propanediol. Crude glycerol derived from biodiesel production has been shown to be toxic to the organism even in low concentrations. By examination of different pretreatments we found that storage combined with activated stone carbon addition facilitated the utilization of crude glycerol. A pH-controlled reactor with in situ removal of butanol by gas stripping was used to evaluate the performance. The fermentation pattern on pretreated crude glycerol was quite similar to that on technical grade glycerol. *C. pasteurianum* was able to utilize 111 g/l crude glycerol. The average consumption rate was 2.49 g/l/h and maximum consumption rate was 4.08 g/l/h. At the maximal glycerol consumption rate butanol was produced at 1.3 g/l/h. These rates are higher than those previously reported for fermentations on technical grade glycerol by the same strain. A process including pretreatment and subsequent fermentation of the crude glycerol could be usable for industrial production of butanol by *C. pasteurianum*.

Keywords Glycerol · Pretreatment · Biofuel · Butanol · Anaerobic fermentation

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Introduction

As a consequence of expanding biodiesel production, the cost and availability of glycerol has changed markedly because 10% glycerol is released during a typical biodiesel production process. This crude glycerol is considered as waste [17] and it is, therefore, important that new and sustainable solutions for utilization of the glycerol are developed [24, 30].

Butanol is an important bulk chemical for a wide range of industrial applications. Recently, its potential as a fuel has been discussed [25]. Butanol can be blended directly with standard oil-based fuels and has a range of advantages over the more commonly used ethanol including a much lower enthalpy of vaporization, lower solubility of water, less corrosiveness combined with a much higher energy density. As a consequence, a 100% replacement of the standard oil-based fuels is possible without structural modifications of the engine. Furthermore, butanol can be blended directly at the refinery for delivery using existing infrastructure [5].

The production of butanol from the biodiesel-derived glycerol will not only constitute a sustainable utilization of waste glycerol for fuel production, but also a means to produce a bulk chemical for use in the chemical industry.

The most studied organism for biological production of butanol is *Clostridium acetobutylicum*. However, *C. acetobutylicum* is unable to grow on glycerol as it cannot reoxidize the excess NADH generated in the cellular glycerol catabolism [7, 8, 29].

Fermentation of glycerol by the related organism *C. pasteurianum* was initially described in 1983 by Nakas et al. [22]. The main products of the energy metabolism are *n*-butanol and 1,3-propanediol (1,3-PDO) together with smaller amounts of ethanol and acetic acid [13, 22].

Only few publications have dealt with optimization of glycerol fermentation by *C. pasteurianum* [2, 3]. Recently, a study demonstrating the possibility of utilizing crude glycerol in fermentative butanol production was reported [28]. This study proved the concept, but extremely slow growth (24 days) and high toxicity of the crude glycerol limited the immediate industrial applicability.

In this study our goal was to optimize the growth conditions of *C. pasteurianum* on the crude glycerol to increase the growth rate and product yield to make the process relevant for industrial application.

Materials and methods

Bacterial strain

The strain *C. pasteurianum* (DSMZ 525) was purchased from the German Collection of Microorganism and Cell Cultures (DSMZ), Göttingen, Germany.

Medium and conditions

For all fermentations the minimal medium described by Biebl [2] was used. The concentration of $FeSO_4$ (7.5 mg/l) was increased to stimulate butanol production as suggested by Dabrock et al. [3]. Batch fermentations were carried out without pH control but supplemented with $NaHCO_3$ (2.6 g/l). All cultures were incubated anaerobically at 37°C at pH 6.0 under a gas phase of N_2/CO_2 (20:80). Cell growth was determined using optical density (OD) at 595 nm. The carbon source was either purified (technical grade) glycerol (Sigma-Aldrich, St. Louis, Missouri, USA) or biodiesel-derived glycerol (crude glycerol) (Meroco, Leopoldov, SK). The crude glycerol was specified to contain 800–850 g/l glycerol, max 1 g/l methanol, 55 g/l NaCl, and 25 g/l MONG (matter organic non-glycerol) by the supplier.

Batches of glycerol

Two different batches of glycerol were used in this study. Batch I was derived from biodiesel production from 90% rape seed oil and 10% spent cooking oil. Batch II was derived from biodiesel production from 100% rape seed oil.

Toxicity test

To assess the inhibition of the substrate, toxicity tests were performed in batch experiments. A series of vials were prepared with different concentrations of glycerol ranging from 10 to 150 g/l. The other media components were kept constant. The vials were inoculated with 0.2 ml of an overnight culture of *C. pasteurianum*. After 20 h of incubation, growth was evaluated on the basis of the amount of dry cell mass. The dry cell mass was determined by establishing a linear correlation between dry cell mass and cell suspension absorbance at 595 nm. All tests were performed in at least duplicate.

Pretreatment of the crude glycerol

Purification by carbonation

pH was raised to 11.5 by stepwise addition of a calcium hydroxide solution (milk of lime) followed by incubation for 3 h at 70°C. Seven grams of solid CO_2 (dry ice) per 50 ml of glycerol was added. After sedimentation of calcium carbonate, the glycerol was cleared by centrifugation at 8,000 rpm at 4°C for 20 min. pH was adjusted to 7.00 by addition of 2 M HCl. Centrifugation was repeated using the aforementioned conditions as further sedimentation occurred after the pH adjustment.

Electrodialysis

The removal of NaCl from the crude glycerol was done by electrodialysis, using a PC Cell ED 64-4 (PC Cell GmbH, Heusweiler, Germany) electrodialysis stack equipped with nine cationic exchange membranes, ten anionic exchange membranes enclosed by two cationic exchange membranes. For each membrane, the active surface area was 64 cm^2 and the flow channel width between two membranes was 0.5 mm. The feeding rate of 50% diluted glycerol was 1.63 l/h. Voltage across the electrodialysis cell was 15 V. K_2SO_4 (0.1 M) was used as the electrode solution and circulated with a flow of 53.1 l/h.

Activated stone carbon

Crude glycerol was treated with activated stone carbon 0.4–0.85 mm (Gert Strand, Malmö, Sweden) after 50% dilution in water. Approximately 20% (w/v) activated stone carbon was added directly to the crude glycerol, followed by static incubation at room temperature for 8 h. The stone carbon was removed from the glycerol by centrifugation at 10,000 rpm for 10 min [10].

The effect of activated stone carbon during growth was tested in batch experiments. Approximately 0.05 g of activated stone carbon was added per 10 ml of culture medium prior to autoclaving.

HPLC analysis

Liquid samples were analyzed for glycerol, lactate, acetate, 1,3-PDO, butyrate, ethanol, acetone, and butanol using

high pressure liquid chromatography. The HPLC system was equipped with a Rezex ROA-Organic Acid column (Phenomenex, Torrance, California, USA) and an RI 101 refractive index detector (Shodex, Kawasaki, Japan). The mobile phase was 4.5 mM H_2SO_4, pumped at a flow rate of 0.6 ml/min. Before the analysis the samples were centrifuged at 10,000 rpm for 10 min. The supernatant was separated and diluted to a suitable concentration range before loading on the HPLC system.

Reactor fermentation

The reactor fermentation was performed in 500-ml glass reactors (active volume 400 ml) with water jackets ensuring a constant temperature of 37°C. The pH was maintained at 6.00 by addition of 1 M KOH managed by a Knick Stratos® controller unit. Mixing was performed by magnetic stirring. The inoculation was done by injecting 40 ml of an overnight culture into the reactor. For removal of the solvent produced, gas stripping was applied circulating the gas phase at a flow rate of approximately 600 ml/min in an Ø15-mm non-gas-permeable tube by means of a peristaltic pump (Fig. 1). The 400-mm condenser (Lenz, Wertheim, Germany) was cooled by ice water. The condensate was collected in a flask and the dehumidified gas was re-injected at the bottom of the reactor [6, 9]. Excess gas was collected in a non-gas-permeable bag. Water was added during the fermentation to ensure a constant volume of the reactor, as butanol and water were removed by the gas stripper.

For stoichiometric calculations the composition of cell biomass was assumed to be $CH_{1.65}N_{0.23}O_{0.45}$ [14].

Results and discussion

Batch experiment

Cell growth

To evaluate the crude glycerol utilization capabilities of *C. pasteurianum*, toxicity tests were performed within the first 2 months after production of the glycerol.

As shown in Fig. 2, increasing concentrations of technical grade glycerol at constant nutrient concentrations did not affect growth of *C. pasteurianum*. This is in accordance with Dabrock et al. [3] who demonstrated that growth by *C. pasteurianum* was not inhibited by glycerol concentrations up to 17%. The crude glycerol, however, appeared to affect growth significantly. Batch I and II glycerol were only utilized to a minor degree. At an initial glycerol concentration at 20 g/l, growth (determined by cell mass dry weight) on batch I glycerol was only 25% relative to the growth on technical grade glycerol, and ceased further as the glycerol concentration increased. Growth on batch II glycerol was almost below the detection level at all tested concentrations. As a result of the high toxicity of both batches of crude glycerol, the use of simple pretreatment methods was investigated in order to optimize the fermentation of the crude glycerol.

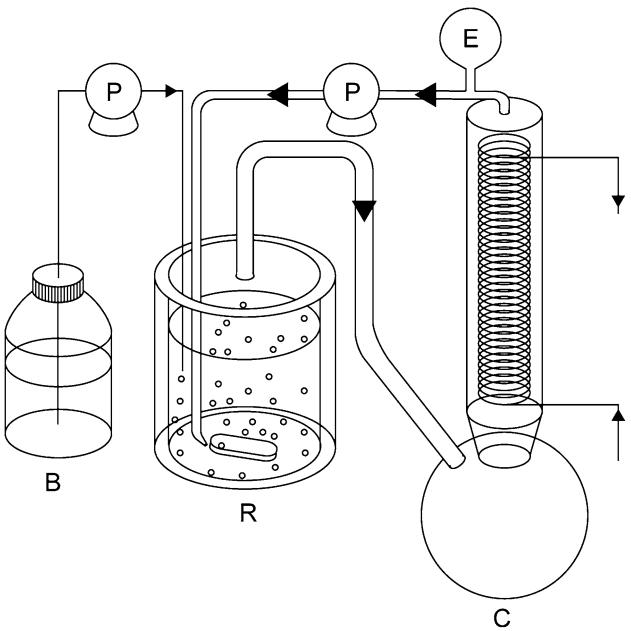

Fig. 1 Experimental setup. *R* reactor equipped with pH control *B*. *C* condenser cooled by ice water, with a collector flask at the bottom. *E* expansion bag collecting gas produced. *P* peristaltic pumps

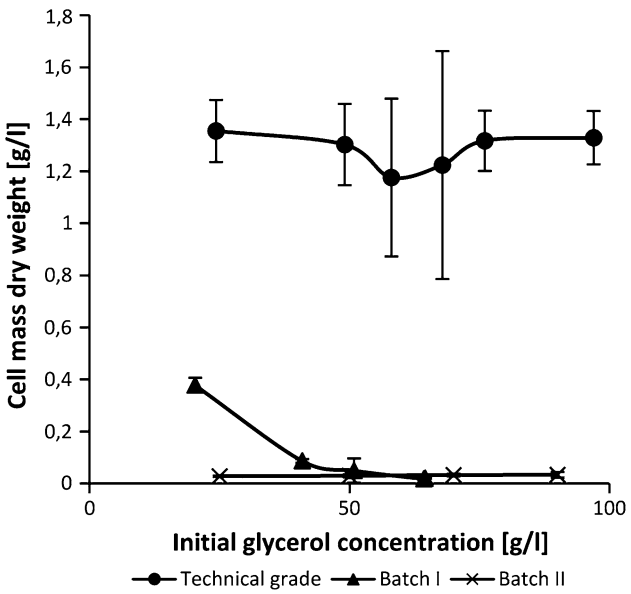

Fig. 2 Growth on the two batches of glycerol, and the technical grade glycerol. Measurements were carried out after 20 h incubation. *Error bars* standard deviations

Pretreatment of the glycerol

Carbonation

The method of carbonation was adapted from the sugar industry, where it is used for purification of the sugar juice. Milk of lime ($Ca(OH)_2$) is added to the sugar juice after which CO_2 is bubbled through the solution. Excess calcium, which has not been consumed during the liming process, reacts with introduced carbon dioxide to form calcium carbonate, which precipitates together with impurities in the carbonation reaction [23]. Also, the increase in pH can chemically change the properties of the inhibitory compounds leading to a reduced toxicity [19]. It has previously been shown that the presence of $CaCO_3$ does not inhibit growth of *C. pasteurianum*, but contributes to stabilize the pH [18].

The experiments were performed on batch I glycerol. At low concentrations, the positive effect of the treatment was evident (Table 1). At initial glycerol concentrations of 18 and 35 g/l, growth was stimulated by 95 and 152% respectively compared to growth on unpurified crude glycerol, representing 68.2 and 41.1% of the growth on technical grade glycerol. At an initial glycerol concentration of 52 g/l, growth was stimulated by 200%.

Although it was possible to increase the tolerance towards the crude glycerol by carbonation, the effect at high glycerol concentrations was limited and we consider the method inadequate as a stand-alone pretreatment process.

Electrodialysis

It has been documented that a related *Clostridium* species (*C. butyricum*) was not able to ferment alfalfa at NaCl concentrations above 5.84 g/l [27]. The concentration of NaCl in the crude glycerol is approximately 55 g/l. In a toxicity test of technical grade glycerol supplemented with 60 g/l NaCl, 50% inhibition at an initial glycerol concentration of 50 g/l was observed (data not shown).

By diluting the crude glycerol from batch II with water (1:1) it was possible to remove 98% of the NaCl from the crude glycerol by electrodialysis. Electrodialysis has previously been applied for purification of crude glycerol and has proven to be economically feasible [26].

The results of the toxicity test of the crude glycerol compared to the dialyzed glycerol appear in Table 1. Removal of NaCl and other salts by electrodialysis had a negative effect on the growth rate, which makes this pretreatment not of interest.

Activated stone carbon

Several investigations have pointed out that activated stone carbon added to substrates containing inhibitory

Table 1 Growth on carbonized crude glycerol, electrodialyzed crude glycerol, activated stone carbon pretreated crude glycerol, and stored glycerol compared to non-treated crude glycerol

Carbonized			Electrodialyzed			Pretreated with activated stone carbon			Storage—batch I			Storage—batch II		
Initial glycerol (g/l)	Growth (g/l)	Effect (%)	Initial glycerol (g/l)	Growth (g/l)	Effect (%)	Initial glycerol (g/l)	Growth (g/l)	Effect (%)	Initial glycerol (g/l)	Growth (g/l)	Effect (%)	Initial glycerol (g/l)	Growth (g/l)	Effect (%)
18	0.90	95.0	25	0.08	−75.1	20	1.06	135.9	27	0.88	133.4	28	0.68	2,341.0
35	0.86	152.0	45	0.01	−83.3	41	0.27	617.6	70	0.15	741.9	54	0.15	414.7
52	0.50	200.0	67	0.01	−59.2	68	0.08	153.3	98	0.08	ND	73	0.04	27.4

The comparison is given as percentage stimulation or inhibition. Measurements were carried out after 20 h of incubation

compounds can act both as an adsorbent of toxic compounds but also as a protective attachment surface and support for the growing microorganisms in the substrate [15, 16, 20]. Habe et al. [10] observed that pretreatment of crude glycerol with activated stone carbon resulted in increased growth of *Gluconobacter* sp. at high initial glycerol concentrations. Furthermore, 90% of the productivity was maintained compared to fermentation done on technical grade glycerol. A similar increase was not seen in our experiments with *C. pasteurianum*, although a certain relief of inhibition was observed compared to untreated crude glycerol (Table 1). The treatment was performed on batch I glycerol. At an initial glycerol concentration of 20 g/l, the growth on detoxified glycerol was 81% of the growth on technical grade glycerol. Compared to untreated crude glycerol, growth increased by 147%. At 41 and 68 g/l, the growth on pretreated glycerol was 20 and 6.1% respectively of the growth on technical grade glycerol. The difference in the toxicity of the pretreated crude glycerol between the study by Habe et al. [10] and our experiments could derive from the different organisms used. The effect of activated stone carbon was better than any of the two other pretreatments but still insufficient as a stand-alone process due to the low efficiency at high glycerol concentrations.

Storage of crude glycerol

Storage of the crude glycerol for 10 months at 20°C had a clear effect on the growth on the crude glycerol (Table 1). In both batches of glycerol growth was stimulated by storage. On batch I glycerol growth was more than doubled at an initial glycerol concentration of 27 g/l compared to the fresh glycerol. At initial glycerol concentration of 70 g/l growth was stimulated by 741% which represents 11.4% of the growth on technical grade glycerol. Growth on batch II glycerol was also stimulated by storage. In fresh glycerol, no growth was detectable, but after storage considerable growth was observed at an initial glycerol concentration of 25 g/l. At 54 and 73 g/l, the growth on pretreated glycerol was stimulated by 414 and 27.4% respectively.

The results clearly show that storage of crude glycerol has a profound effect on toxicity. The most obvious reason for this would be chemical changes in the crude glycerol which contains many other compounds besides glycerol.

An analysis of the change in the glycerol composition upon storage could indicate which compound(s) was (were) responsible for the observed inhibition in the fresh glycerol. A comparison of the HPLC chromatograms from the samples before and after storage indicates that chemical changes occur upon storage (Fig. 3). However, the storage primarily resulted in the appearance of new peaks, which were not detected in the fresh glycerol. Only one peak disappeared after storage (at 13 min retention time). Since the peak at 13 min appears in the batch I sample upon storage, it is not likely to be the decisive factor for inhibition.

Combination of storage and activated stone carbon

Among the four pretreatments, activated stone carbon and storage were the most efficient, but not satisfactory as stand-alone processes. We therefore decided to combine the two treatments, but also to exploit the demonstrated benefits of activated stone carbon as an attachment surface for bacteria. The experiment was carried out on stored glycerol from both batch I and batch II. The results from the experiments are shown in Fig. 4.

On batch II, the addition of activated stone carbon stimulated growth by an approximate factor of 13 at 73 and 98 g/l glycerol compared to stored crude glycerol without activated stone carbon but still only constituted 39% of the growth achieved on technical grade glycerol. On batch I, the treatment of the stored crude glycerol had even more profound effects. The effect of the treatment is a visibly denser culture and high amounts of foam in the culture tube with activated stone carbon (Fig. 5).

At an initial concentration of 70 g/l the stored glycerol supported a growth of 11% of the technical grade glycerol whereas 154% growth was observed when activated stone carbon had been added. At 98 g/l stored glycerol growth constituted 68% compared to the technical grade glycerol when activated stone carbon was added. This was 23 times higher than stored crude glycerol at the same concentration.

Even at 118 g/l, limited growth was observed when activated stone carbon was added to the stored crude glycerol.

Fermentation

Technical grade glycerol

To evaluate the product profile and the fermentation capabilities of *C. pasteurianum*, a batch reactor fermentation on technical grade glycerol was performed. Two reactors were run simultaneously, one with and one without gas stripping. Both reactors had an initial glycerol concentration of approximately 100 g/l, which has been shown not to inhibit growth [2, 3].

The concentrations of substrate and products as a function of time are shown in Fig. 6. An initial lag phase of 15 h was seen in both fermentations. The highest consumption rate was observed after 21 h in both reactors. In the fermentation without gas stripping, glycerol was

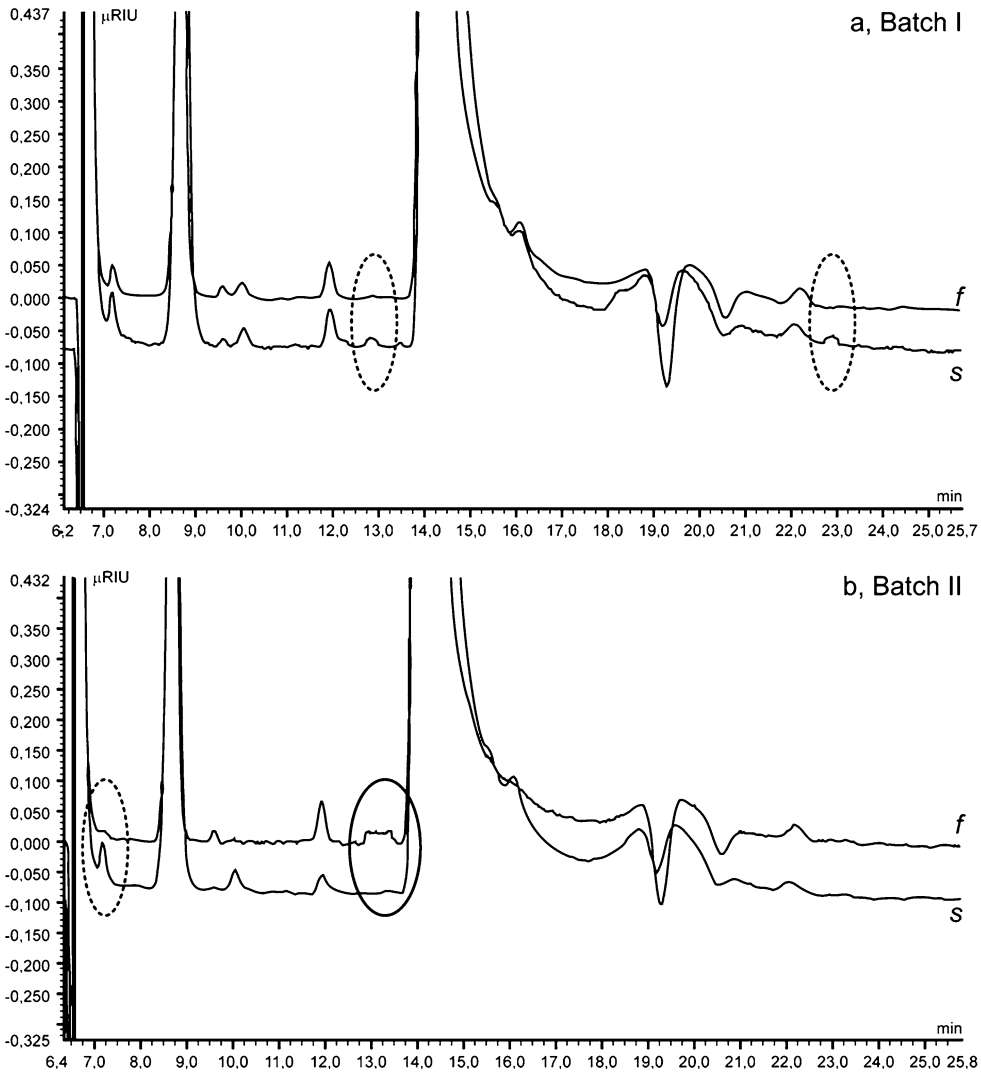

Fig. 3 Chromatograms of the HPLC analyses of the batch I and II glycerol before and after 10 months of storage. *f* fresh sample, *s* stored sample, *dotted ovals* peaks that appear after storage, *unbroken oval* peak that disappears after storage

consumed at a rate of 6.1 g/l/h. Butanol was produced at a rate of 1.2 g/l/h and 1,3-PDO at a rate of 1.1 g/l/h (data not shown). In the fermentation with gas stripping, the glycerol was consumed at a rate of 4.94 g/l/h along with the production of 1,3-PDO at a rate of 0.64 g/l/h. The butanol production rate could not be estimated because butanol was continuously removed by the gas stripping at an unknown rate.

During the active 45 h of fermentation, 60 g/l glycerol was consumed in the reactor without gas stripping, whereas almost all the glycerol (103 g/l) was utilized in the reactor with gas stripping. This was most probably due to the continuous removal of butanol by gas stripping, which relieved the inhibition of *C. pasteurianum*, which has also been observed in experiments with acetone, butanol, and ethanol fermentation [6, 9].

In Table 2, the mass balances of the two fermentations are shown. Only metabolized glycerol is considered, and both fermentations are normalized to 1,000 mM glycerol converted. The production of CO_2, H_2, and H_2O was calculated from the stoichiometry of the reactions and not supported by measurements. In both fermentations the production of lactate was very low and acetone and acetate were barely measurable. This fermentation pattern resembles previously described *C. pasteurianum* fermentations [2, 3, 28].

In the reactor with gas stripping the concentrations of butyrate and acetate were higher compared to the fermentation without gas stripping. A residual acidogenic activity or an incomplete uptake of the acids, occurring during the biphasic fermentation exhibited by the clostridia, could explain the higher acid concentration [1, 11, 12]. It should be noted that the production of butyrate and acetate yields ATP in contrast to the production of 1,3-PDO, ethanol, or butanol [21]. Therefore, the observed acid production could be due to the need for more ATP during the prolonged growth in the gas-stripped reactor.

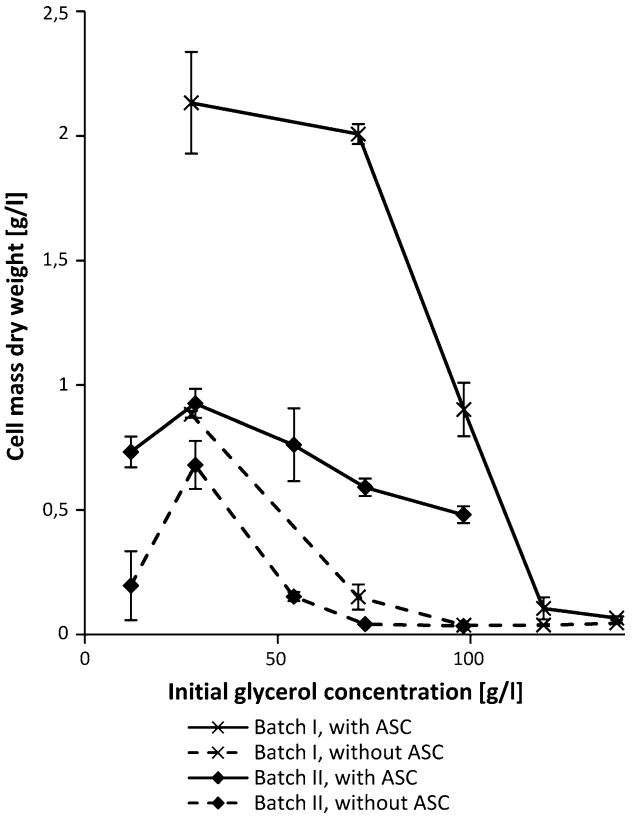

Fig. 4 Effect of adding activated stone carbon (ASC) to stored batch I and batch II glycerol. Measurements were carried out after 20 h of incubation. *Error bars* standard deviations

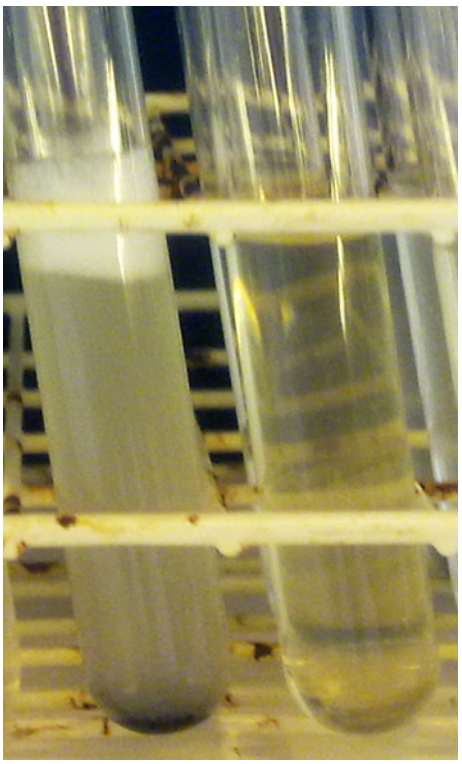

Fig. 5 Anaerobic culture tubes with an initial glycerol concentration of 98 g/l stored crude glycerol (batch I) after 20 h of incubation. Activated stone carbon was included in the *left tube*

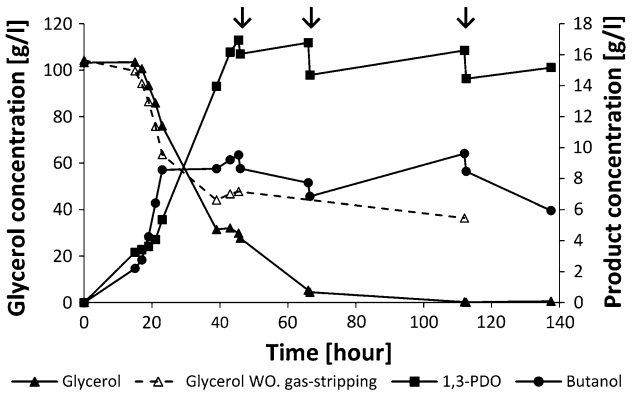

Fig. 6 Profile of the fermentation using technical graded glycerol showing concentrations of glycerol and products as a function of the time. *Arrows* points at which water was added

Stored crude glycerol with activated stone carbon

To test the positive effects achieved by active stone carbon addition to the stored crude glycerol in the 10-ml batch experiments, a reactor experiment was carried out combining activated stone carbon addition and gas stripping. The most toxic stored batch II crude glycerol was used for the experiment (Fig. 7) since this left a bigger potential for process optimization than the stored batch I crude glycerol.

Initially, an 8-h lag phase occurred before the consumption of glycerol began, which was shorter than that observed for technical grade glycerol. Gas stripping was started after 19.75 h and 3 h later the butanol reached a concentration of 10.8 g/l which arrested the metabolism of *C. pasteurianum*. When gas stripping had reduced the butanol concentration below 6 g/l after 45 h, the activity was resumed. After 51 h, glycerol was supplemented without any immediate effect on the fermentation. The activity continued until the butanol concentration had increased to more than 9 g/l and did not resume although the concentration was reduced to below 5 g/l. At the end of the fermentation a residual glycerol concentration of 35 g/l was left in the broth.

Since the activity was unaffected by glycerol addition at $t = 51$ h and the activity did not resume when the butanol concentration was reduced below the toxicity level, a lack of nutrients could have caused the discontinuation of the fermentation.

Overall, the fermentation persisted for 70 h utilizing 111 g/l (1.21 M) of the glycerol, including 8 h of lag phase and a butanol-inhibited period of 19 h. Excluding the lag phase and the inhibited period, the average glycerol consumption rate was 2.49 g/l/h and the productivities were 0.42 g/l/h for 1,3-PDO and 0.68 g/l/h for butanol. The highest rates were observed in the period from 19.75 to

Table 2 Mass balances in mmol of the fermentation of technical grade glycerol with and without gas stripping (GS) and stored glycerol with gas stripping

	Lactate	1,3-PDO	Acetate	Ethanol	Butyrate	Butanol	CO_2	H_2	H_2O	Cell mass[a]	Carbon-recovery (%)
Technical grade glycerol with GS	5	211	27	160	17	223	670	553	408	253	97
Technical grade glycerol without GS	3	205	1	143	0	263	674	470	465	320	99
Stored glycerol with GS	5	169	25	57	0	280	644	480	423	204	88

Balances were normalized to 1,000 mM glycerol

[a] Cell mass calculations were based on the theoretical composition of the biomass as described in the "Materials and methods" section

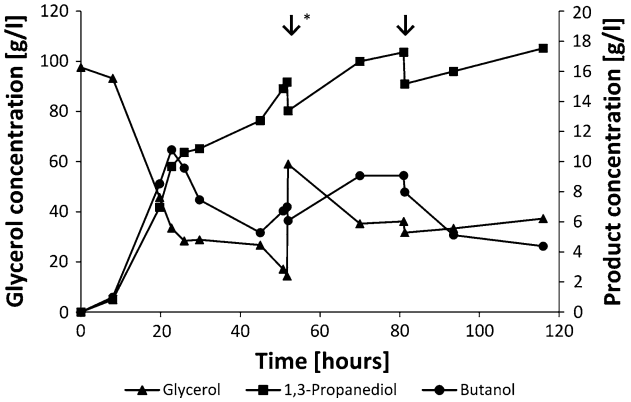

Fig. 7 Profile of the fermentation of stored crude glycerol supplemented with activated stone carbon. *Arrow* point at which water was added, *arrow with asterisk* point at which glycerol was added

22.75 h. In this period glycerol was consumed at 4.08 g/l/h, 1,3-PDO was produced at 0.91 g/l/h, and by correction for the stripping of butanol (when glycerol consumption was inhibited, from 26 to 29.75 h) the butanol productivity was calculated to 1.3 g/l/h. Compared to the maximum rate observed for technical grade glycerol, a slight decrease of 16% in the glycerol consumption rate and an increase of 50% in the 1,3-PDO production rate were observed.

The yields of 1,3-PDO and biomass were lower on stored crude glycerol than on technical grade glycerol. This correlates well with Biebl [2] who suggested that biomass and 1,3-PDO yields go together in glycerol fermentation since glycerol is more reduced than the biomass formed during the fermentation. Surplus reducing equivalents produced during growth are then regenerated by glycerol reduction to 1,3-PDO. The reduced 1,3-PDO yield on stored crude glycerol might, therefore, be a consequence of a lower production of biomass.

Conclusion

During the fermentation of stored crude glycerol using gas stripping for removal of butanol and addition of activated stone carbon it was possible to achieve productivities close to those achieved on technical grade glycerol. The peak glycerol consumption rate was slightly lower for crude glycerol, but the overall fermentation time was similar. On the other hand, the butanol yield was slightly higher when fermenting crude glycerol compared to technical grade glycerol.

In Table 3 our data from the two fermentations are compared with previously reported results from glycerol fermentation with *C. pasteurianum*. As pointed out above, the two fermentations with gas stripping carried out in this study are very similar. Compared to the highest amount of glycerol utilization previously reported [2], we achieved a

Table 3 Comparison of the fermentation data obtained in this study with data from other studies

	Technical grade glycerol with gas stripping	Crude glycerol + activated stone carbon with gas stripping	Technical grade glycerol [2]	Technical grade glycerol [3]	Crude glycerol [28]
Glycerol utilized (g/l)	101	111	63.6	28	22[c]
Time	45 h[a]	43 h[a]	17 h[b]	ND	14 days[c]
Glycerol maximum rate (g/l/h)	4.94	4.08	2.62[b]	ND	0.125[c]
Average glycerol rate (g/l/h)	2.20[a]	2.49[a]	2.33[b]	ND	0.07[c]

ND not determined

[a] Average was calculated by subtraction of the lag phases

[b] Data are derived from a fermentation of 41 g/l glycerol and were extracted from the graph (lag phases were subtracted)

[c] Data were extracted from the figure of the fermentation data (lag phases were subtracted)

75% increase utilizing crude glycerol and 58% increase utilizing technical grade glycerol. The maximum rate in that study was only 57% of our rate. Only one report has dealt with the fermentation of crude glycerol. In that study, only 22 g/l crude glycerol was utilized in 24 days (including 10 days of inhibition) of incubation resulting in a consumption rate of 0.07 g/l/h [28]. The average rate of crude glycerol fermentation achieved in our study is 38 times higher and the maximum consumption rate is 33 times higher. In a review of next-generation biofuels, Dellomonaco et al. [4] state that the low growth rates reported by Taconi et al. [28] limit the immediate industrial applicability of crude glycerol fermentation to butanol. Our study clearly demonstrates that it is possible to ferment crude glycerol derived from biodiesel production at high concentrations and at industrially relevant rates.

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