Bioresource Technology Reports 10 (2020) 100408

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.journals.elsevier.com/bioresource-technology-reports

High rate 2,3-butanediol production with *Vibrio natriegens*

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ARTICLE INFO

Keywords:
Vibrio natriegens
High productivity
2,3-Butanediol
Elemental biomass composition
Chemostat cultivations
Microaerobic conditions

ABSTRACT

The aim of this study was to investigate if the high substrate turnover rates of *Vibrio natriegens* enable 2,3-butanediol production at high productivities. Introduction of a heterologous pathway led to 2,3-butanediol production at yields comparable to *Escherichia coli* using glucose, sucrose and sugar beet molasses. Microaerobic fed-batch cultivations maintained at 0–1% dissolved oxygen yielded a combined diol titer of 49.9 g L^{-1} of 2,3-butanediol and acetoin and a production rate of 3.9 g L^{-1} h^{-1}. Optimization of the oxygen supply in chemostat cultivations increased the product yield to 70% of the theoretical maximum at volumetric and specific production rates of 2 g L^{-1} h^{-1} and 0.66 g g^{-1} h^{-1}, respectively. In conclusion, this study could for the first time demonstrate *V. natriegens* as novel host for 2,3-butanediol production with high volumetric and specific productivity, underlining its potential for microbial chemical production.

1. Introduction

Economic production of biofuels and chemicals using microbial cell factories as sustainable alternative to chemical production remains challenging. Despite the availability of advanced techniques for metabolic engineering of microorganisms, high fermentation costs caused by expensive feedstocks or media supplements (e.g. yeast extract) often limit the application of microbial cell factories (Nielsen and Keasling, 2016; Stephanopoulos, 2007). Thus, high titer, rate and yield are prerequisites for implementation of microbial fermentation processes on an industrial scale (Van Dien, 2013). Since high production rates are associated with high growth rates, fast-growing organisms have gained great attention as microbial hosts. Recently, research in the gram-negative bacterium *Vibrio natriegens* has been revived more than 60 years after its first description (Eagon, 1962; Payne et al., 1961) due to its exceptionally fast doubling time of less than 9.4 min (Hoffart et al., 2017). This striking feature makes *V. natriegens* a potentially valuable chassis not only for biotechnological processes but also as cloning host to accelerate cloning procedures compared to *Escherichia coli* as traditional cloning host (Calero and Nikel, 2019).

V. natriegens has been fully sequenced (Lee et al., 2016; Maida et al., 2013), tools for genetic manipulations have been developed (Lee et al., 2019, 2016; Weinstock et al., 2016), natural transformation has been demonstrated (Dalia et al., 2017) and prophage-free strains have been generated (Pfeifer et al., 2019). Moreover, the ability to secrete proteins to the culture medium (Weinstock et al., 2016) and the application of *V. natriegens* in cell-free protein synthesis systems was shown (Failmezger et al., 2018; Wiegand et al., 2019), indicating potential for recombinant protein production.

^{13}C-flux analysis revealed a central carbon metabolism similar to *Escherichia coli* (Long et al., 2017) and high substrate uptake rates under aerobic and anaerobic conditions have been described (Hoffart et al., 2017). As a result, the potential of *V. natriegens* as a host for chemical production has been demonstrated by enhancing formation of poly-β-hydroxybutyrate and L-alanine (Dalia et al., 2017; Hoffart et al., 2017) and for the production of natural products such as melanin (Wang et al., 2019), beta-carotene and violacein (Ellis et al., 2019).

2,3-Butanediol is of interest for a broad range of applications in the chemical industry (e.g. as a precursor for synthetic rubber production) (Song et al., 2019). In addition to natural producers, several studies have successfully constructed recombinant hosts like *E. coli* for 2,3-butanediol production (Erian et al., 2018; Hwang et al., 2018; Xu et al., 2014). Generally, 2,3-butanediol formation in natural and engineered hosts can be optimized by oxygen supply, with microaerobic conditions favoring 2,3-butandiol over other fermentation products (anaerobic conditions) or respiration (aerobic conditions). Three enzymes are required for synthesis of 2,3-butanediol from pyruvate, (i) α-acetolactate synthase, (ii) acetolactate decarboxylase and (iii) butanediol dehydrogenase. The overall reactions equation is:

$$2\ \text{Pyruvate} + \text{NADH} + \text{H}^{+} \rightarrow 2,3-\text{butanediol} + 2\ \text{CO}_2 + \text{NAD}^{+}.$$

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https://doi.org/10.1016/j.biteb.2020.100408
Received 29 January 2020; Received in revised form 23 February 2020; Accepted 25 February 2020
Available online 27 February 2020

The reaction equation for 2,3-butanediol synthesis shows that anaerobic conditions with 2,3-butanediol production are infeasible, because the 2 $NADH + H^+$ generated by glycolysis cannot be fully re-oxidized.

Yet, none of the processes with engineered *E. coli* has achieved product formation rates high enough for economic synthesis of 2,3-butanediol.

The aim of this study was to investigate if the high substrate turnover rates of the fast-growing organism *V. natriegens* enable 2,3-butanediol production at high productivities. To achieve this goal, (I) a plasmid-based heterologous pathway for 2,3-butanediol, that allows high titer production in *E. coli* W, was introduced into *V. natriegens*. (II) The results of batch, fed-batch and chemostat cultivations using glucose, sucrose and sugar beet molasses as substrates gave insights into strain physiology under production conditions and the influence of oxygen supply on 2,3-butanediol formation. (III) Comparing the titers, rates and yields obtained here with data available for *E. coli* demonstrate high rate 2,3-butanediol production in *V. natriegens* for the first time and underlines the potential of *V. natriegens* as novel host for microbial chemical production.

2. Materials and methods

2.1. Strain and media

Vibrio natriegens DSM 759 (= ATCC 14048) was used in this study. Miller lysogeny broth (LBM) contained per liter: 10 g tryptone, 5 g yeast extract and 10 g NaCl. Enhanced 2xYT (e2xYT) contained per liter: 32 g tryptone, 20 g yeast extract, 17 g NaCl, 0.2% (w/v) glucose and 17.6 mM Na_2HPO_4, pH 7.4. Chemically defined medium as described previously, additionally containing 15 g L^{-1} NaCl, was used for all cultivations (Erian et al., 2018). Carbon sources and yeast extract were added from sterilized stock solutions as indicated.

2.2. Cultivations

V. natriegens was streaked on LBM plates containing 250 μg mL^{-1} kanamycin and a single colony was used to inoculate 10 mL LBM. After 6 h, 250 mL LBM were inoculated with 2 mL of this culture and incubated overnight. Liquid precultures were grown at 30 °C and 200 rpm.

Small scale cultivations were started by inoculating 20 mL LBM in 100 mL shake flasks with an OD_{600} of 0.5 and incubated at 37 °C and 200 rpm. Bioreactor cultivations were performed in four parallel DASGIP® Benchtop Bioreactors (Eppendorf AG, Hamburg, Germany) at 37 °C. The working volume was 1 L for fed-batch and 650 mL for chemostat cultivations. All cultivations were maintained at pH 7.0 by addition of NH_4OH (12.5% v/v) and were monitored by a pH electrode (Mettler-Toledo GmbH, Giessen, Germany). The dissolved oxygen was monitored by a VisiFerm DO 225 probe (Hamilton, Reno/NV, USA) and was maintained in fed-batch cultivations at 0–1% DO by adjusting the stirrer speed at a constant gassing rate of 1 vvm. For chemostats, a stirrer speed of 1000 rpm was used, and the gassing rate and gas composition was as indicated. Offgas analysis (CO_2 and O_2 concentrations) was carried out using the off-gas analysis module GA4 (Eppendorf AG, Hamburg, Germany).

2.3. HPLC and biomass analysis

Culture supernatant was analyzed by HPLC with an Ultimate 3000 system (Thermo Scientific, Waltham/MA, USA) using an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, Hercules/CA, USA), a refractive index detector (Refractomax 520, Thermo Scientific, Waltham/MA, USA) and an UV detector (Ultimate 3000, Thermo Scientific, Waltham/MA, USA) as described previously (Erian et al., 2018).

Cell dry weight of bioreactor cultivations was determined gravimetrically in triplicates by weighing 4 mL culture broth after washing and drying. Elementary biomass composition of *V. natriegens* ($C_{1.000}H_{1.770}O_{0.607}N_{0.153}P_{0.015}S_{0.003}$, C-content = 45% (w/w)) was determined as described previously (Novak et al., 2018).

2.4. Transformation of V. natriegens

A single colony was inoculated into 10 mL e2xYT. After 4 h, 100 mL prewarmed e2xYT were inoculated with 2 mL of this starter culture grown until an OD_{600} of 0.4–0.6 (~ 1.5 h) was reached. Cultures were grown at 28 °C and 200 rpm. The culture was washed three times with electroporation buffer (680 mM sucrose, 7 mM K_2HPO_4, pH 7.0, (Weinstock et al., 2016)) and resuspended in residual buffer. 50 μL cell suspension was transformed with 1 μL plasmid DNA (= 20 ng) or H_2O as negative control in 4 mm cuvettes (1800 V, 200 Ω and 25 μF). Transformations were recovered in 1 mL fresh and prewarmed e2xYT containing 680 mM sucrose for 1.5 h at 30 °C under constant shaking and plated on LBM plates containing 100 μg mL^{-1} kanamycin which were incubated overnight at 30 °C. Positive transformants were identified by plasmid DNA isolation and restriction digests.

3. Results and discussion

3.1. Constructing a 2,3-butanediol producing strain of Vibrio natriegens

Vibrio natriegens shows a high degree of compatibility with genetic elements used for *E. coli* (Weinstock et al., 2016). Therefore, in this study, it was attempted to introduce a heterologous production pathway for 2,3-butanediol into *V. natriegens* using an expression plasmid (p445_Ediss) enabling efficient 2,3-butanediol production in *E. coli* W (Erian et al., 2018). p445_Ediss is a high copy number vector containing a pUC ori and a kanamycin resistance cassette (plasmid map, Additional File 1, Fig. S1). Additionally, three genes from *Enterobacter cloacae* subsp. *dissolvens*, a natural producer of 2,3-butanediol, namely acetolactate synthase (*budB*), acetolactate decarboxylase (*budA*) and 2,3-butanediol dehydrogenase (*budC*) are individually expressed from constitutive promoters (J23114 for *budB* and *budA* and J23105 for *budC*). It was evaluated if introducing p445_Ediss enables 2,3-butanediol production in *V. natriegens*, given that the constitutive promoters from the Anderson library also drive gene expression in *V. natriegens*.

3.2. Small scale production of 2,3-butanediol from different carbon sources

Upon successful introduction of p445_Ediss and an empty vector control (VC), yielding *V. natriegens* 445_Ediss and VC, one transformant each was selected to test if *V. natriegens* is able to produce 2,3-butanediol. To that end, 445_Ediss and VC were cultivated in shake flasks using defined minimal medium containing 50 g L^{-1} glucose.

2,3-Butanediol and acetoin were detected as the main fermentation products (Table 1) in *V. natriegens* 445_Ediss, whereas the VC culture incompletely consumed glucose and did not produce any 2,3-butanediol or acetoin (data not shown). This shows that the ability to produce 2,3-butanediol is depending on the presence of plasmid 445_Ediss and that the promoters of the Anderson promoter library, designed for constitutive gene expression in *E. coli*, are also functional in *V. natriegens*.

The consumption rate of glucose was approximately double to what was observed for *E. coli* and the medium was depleted of glucose after 13 h. This represents a significant rate enhancement already in small scale shake flask cultivations. A combined diol yield (2,3-butanediol plus acetoin) of 0.39 g g^{-1} for glucose was obtained which is 78% of the theoretical maximum. This yield is comparable to values previously reported for *E. coli* BL21 using glucose in semi-defined medium containing yeast extract (0.46 g g^{-1}, Xu et al., 2014) and to values obtained with construct 445_Ediss in *E. coli* W using glucose in defined minimal medium (0.39 g g^{-1}, Erian et al., 2018). In addition, small

Table 1
Small scale shake flask cultivations of *V. natriegens* 445_Ediss using 5% (w/v) glucose, sucrose and sugar beet molasses as substrates in defined minimal medium. Cultivation time depended on complete substrate depletion, and was 13, 20 and 24 h for glucose, sucrose and sugar beet molasses, respectively. Errors are given as standard deviations from three independent biological replicates. $Y_{diol/S}$: sum of 2,3-butanediol and acetoin [g] per substrate [g], $Y_{by\text{-}product/S}$: sum of acetic, succinic, formic, lactic and pyruvic acid and ethanol [g] per substrate [g].

Carbon source	Substrate consumed [g L^{-1}]	OD_{600} [−]	2,3-Butanediol [g L^{-1}][a]	Acetoin [g L^{-1}][a]	Acetic acid [g L^{-1}]	Succinic acid [g L^{-1}]	Formic acid [g L^{-1}]	Lactic acid [g L^{-1}]	Ethanol [g L^{-1}]	Pyruvic acid [g L^{-1}]	$Y_{diol/S}$ [g g^{-1}]	$Y_{by\text{-}product/S}$ [g g^{-1}]
Glucose	43.04 ± 1.76	6.57 ± 0.30	13.38 ± 1.31	3.59 ± 0.30	0.92 ± 0.28	0.29 ± 0.07	0.24 ± 0.10	0 ± 0	0.77 ± 0.06	0.23 ± 0.18	0.39 ± 0.01	0.06 ± 0.01
Sucrose	40.63 ± 2.23	5.87 ± 0.13	8.62 ± 1.94	3.45 ± 0.60	1.00 ± 0.05	0.39 ± 0.14	0.14 ± 0.10	0.09 ± 0.14	2.04 ± 0.55	1.72 ± 0.66	0.30 ± 0.02	0.13 ± 0.01
Sugar beet molasses	50.69 ± 0.67	2.81 ± 0.37	15.05 ± 0.42	6.61 ± 0.22	4.01 ± 0.83	2.46 ± 0.37	0 ± 0	0 ± 0	0 ± 0	n.d.	0.43 ± 0.01	0.13 ± 0.02

n.d. not detected.
[a] It should be noted that the mixture of 2,3-butanediol and acetoin that has been obtained only depends on the availability of NADH and is not a sign of insufficient pathway function. Under glucose depleted conditions at the end of the cultivation, NADH levels are low and formation of acetoin from 2,3-butanediol is used for NADH provision as described previously for *E. coli* (Erian et al., 2018).

amounts of acetic acid and ethanol were detected ($Y_{by\text{-}product/S}$ = 0.06 g g^{-1}) (Table 1).

Next, sucrose and sugar beet molasses were tested as carbon sources for 2,3-butanediol production. Both substrates supported growth, and total diol titers of 12.1 and 21.7 g L^{-1} for sucrose and sugar beet molasses, respectively, were obtained. The yield for sugar beet molasses was close to the theoretical maximum, indicating that sugar beet molasses is a suitable substrate for growth and production using *V. natriegens*. Additionally, the higher yield indicates that sugar beet molasses contains valuable other nutrients which therefore do not need to be synthesized de novo and enable higher product yields compared to minimal medium containing only one carbon source.

3.3. Microaerobic fed-batch cultivations enable high rate production of 2,3-butanediol

Based on the promising results obtained in shake flask cultivations, it was next sought to investigate the potential of *V. natriegens* for 2,3-butanediol production under defined conditions. To that end, pulsed fed-batches were used as previously successfully applied for *E. coli* (Erian et al., 2018, 400 rpm and 1 vvm to maintain dissolved oxygen between 0 and 1%). After an aerobic batch phase for biomass production in a medium containing 50 g L^{-1} glucose with or without 5 g L^{-1} yeast extract, glucose was repeatedly pulsed to the culture under microaerobic conditions (see Materials and methods section) which favor 2,3-butanediol formation.

Cultures with and without yeast extract showed a laq phase of about 2 and 5 h, respectively. Subsequently, both cultures displayed rapid substrate consumption, completely consuming 50 g L^{-1} glucose within 5 and 7 h (for cultures with and without yeast extract, respectively) (Fig. 1). These values correspond to maximum specific growth rates and substrate uptake rates of 0.88 h^{-1} and 24.7 g L^{-1} h^{-1} for the culture with yeast extract, and 0.89 h^{-1} and 16.1 g L^{-1} h^{-1} for the culture without yeast extract. Biomass yields were 0.25 and 0.15 g g^{-1} for the cultures with and without yeast extract.

Upon switching to microaerobic conditions, glucose was repeatedly pulsed to the cultures whenever glucose was depleted. During the production phase, rapid glucose consumption rates of 20.0 and 12.2 g L^{-1} h^{-1} for the culture with and without yeast extract were observed (Table 2). Final diol titers of 49.9 g L^{-1} (yeast extract, Fig. 1a) and 27.4 g L^{-1} (no yeast extract, Fig. 1b) were achieved within 12 and 10 h, corresponding to average diol formation rates of 3.88 and 2.67 g L^{-1} h^{-1} (Table 2). Substrate uptake and product formation rates obtained here are significantly higher than what has been described for *E. coli* (Table 2, Erian et al., 2018; Hwang et al., 2018; Xu et al., 2014), underlining the potential of *V. natriegens* as microbial cell factory.

Based on these findings, yeast extract does not seem to be essential for 2,3-butanediol production but appears to improve overall culture fitness rather than improving conversion efficiency (44% for both cultures, Table 2). Despite high diol formation rates and a reasonable diol titer for cultures supplemented with yeast extract, the overall conversion efficiency was rather poor for microaerobic conditions. This low conversion efficiency is reflected by a low diol yield, which is a direct result from mixed acid fermentation products. For the culture with yeast extract the main by-products were succinic and acetic acid (17.6 and 5.3 g L^{-1}). The culture without yeast extract produced a mixture of succinic, acetic and pyruvic acid (7.9, 8.2 and 8.1 g L^{-1}). Furthermore, cultures without yeast extract displayed a high degree of variability and were particularly sensitive to glucose starvation between pulses. A starvation even for a very short time led to metabolic arrest and glucose was not metabolized anymore. In order to avoid such interruptions and to study the diol yield as a function of oxygen supply, chemostat cultivations were used to further characterize 2,3-butanediol formation capacity of *V. natriegens*.

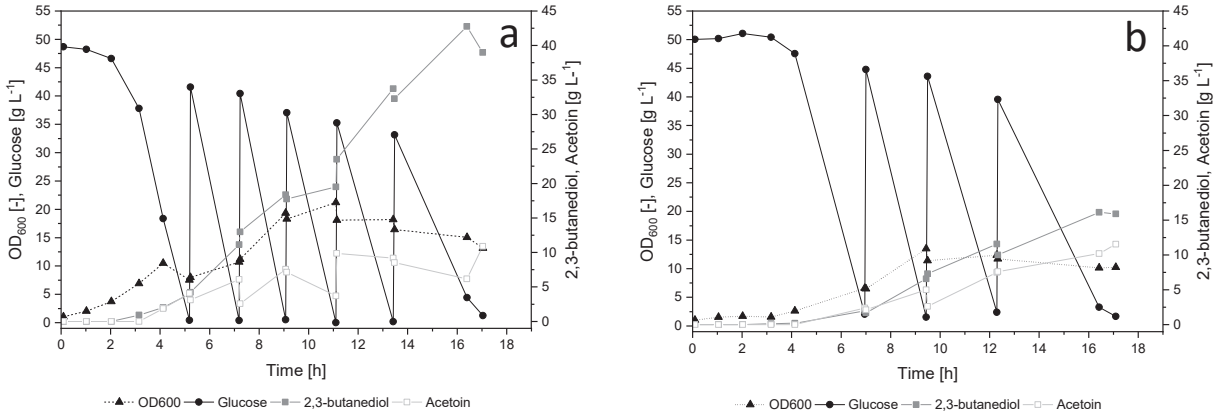

Fig. 1. Microaerobic pulsed fed-batch cultivations of *V. natriegens* 445_Ediss a) with yeast extract and b) without yeast extract. Initial yeast extract concentration was 5 g L^{-1}, the feed medium contained 800 g L^{-1} glucose and a) 5 g L^{-1} yeast extract and b) no yeast extract. Stirrer speed was initially set to 1400 rpm at 1 vvm gassing rate with air. Subsequently, stirrer speed was adjusted to maintain dissolved oxygen levels of 0–1%. Cultures without yeast extract displayed a high degree of variability between cultivations. It is noted that all cultures have been carried out in duplicates and one culture is shown as an example for reasons of clarity. Duplicate cultivations are shown in Additional File 1, Fig. S2.

3.4. Chemostat cultivations reveal the influence of oxygen on the metabolic profile

Chemostat cultivations were used to study overall 2,3-butanediol production efficiency of *V. natriegens* as a function of oxygen supply, dilution rate and carbon source. To initiate continuous cultivations, defined minimal medium with 20 g L^{-1} glucose was fed at a dilution rate of 0.313 h^{-1} to aerobically grown batch cultures of *V. natriegens*. A gassing rate of 0.25 vvm and an agitation rate of 1000 rpm were used as reference point to ensure microaerobic conditions required for 2,3-butanediol synthesis. Under these conditions, low diol formation occurred whereas a concurrent high biomass yield indicated that these conditions favored biomass rather than metabolite formation (Table 3).

Therefore, a second oxygen supply strategy was evaluated by increasing the total gassing rate to 1 vvm and lowering the oxygen content of the gas stream to 5%. It was hypothesized that by applying this strategy, oxygen uptake by the culture would be decreased and more microaerobic conditions can be obtained. Indeed, under these conditions the biomass yield was decreased by 58% to 0.17 g g^{-1} and in turn, diol formation increased by 262% to 6.53 g L^{-1}. This titer corresponds to a yield of 0.35 g g^{-1}, 70% of the theoretical maximum. The specific glucose uptake and diol formation rate under these conditions increased by 141 and 725% to 1.88 and 0.66 g g^{-1} h^{-1}, respectively. Thus, exceptionally high diol production rates were obtained that are 4.4-fold higher than the highest value reported for *E. coli* (Table 2).

The ratio between 2,3-butanediol and acetoin increased 2-fold compared to the high oxygen culture (Table 3), but significant amounts of acetoin were still produced. The low ratio indicates an insufficient availability of NADH. In *E. coli*, it was found that acetoin formation is virtually abandoned under conditions where NADH regeneration depends on 2,3-butanediol synthesis (i.e. knock-out of mixed acid fermentation pathways and low oxygen supply) (Erian et al., 2018). Similar results were obtained for *Bacillus licheniformis*, where high oxygen transfer rates increased acetoin formation and the optimal oxygen transfer rate was determined as 11 to 13 mmol L^{-1} h^{-1} (Heyman et al., 2019). For the low oxygen condition of *V. natriegens* 445_Ediss, a total by-product yield of 0.08 g g^{-1} was observed. This yield is 64% lower compared to *E. coli* W 445_Ediss (0.22 g g^{-1}) but 2-fold higher compared to *E. coli* W Δ*adhE* Δ*ldhA* Δ*pta* Δ*frdA* 445_Ediss (0.04 g g^{-1}) cultivated in pulsed fed-batch cultivations which did not show acetoin formation (Erian et al., 2018). Therefore, strain optimization of *V. natriegens* could be used to reduce by-product formation, to further improve overall diol production and to increase the ratio between 2,3-butanediol and acetoin.

Regardless of potential improvements enabled by strain optimization, it was next attempted to enhance 2,3-butanediol productivity by increasing the glucose concentration in the feed or by increasing the dilution rate at the low oxygen condition. However, in both cases this led to residual glucose in the harvest medium which was also observed when the oxygen supply was further decreased (5 to 2%) (data not shown). These observations could indicate limited metabolic capacity of *V. natriegens* under the conditions tested which may not allow higher substrate uptake rates. To overcome these limitations, cell retention systems could be applied to decouple productivity from growth to achieve high biomass concentrations and productivities.

Finally, untreated sugar beet molasses was tested using the low oxygen condition. $Y_{X/S}$ increased 53% and $Y_{diol/S}$ decreased by 32% (corresponding to 48% of the theoretical maximum) compared to the glucose culture (Table 3). Additionally, a high diol ratio favoring 2,3-butanediol formation was observed, which might be because of additional compounds in molasses increase intracellular NADH availability. Taken together, this shows that *V. natriegens* can also convert sucrose from a complex substrate stream into 2,3-butanediol at reasonable rates and yields.

4. Conclusion

This study could for the first time demonstrate *V. natriegens* as novel host for 2,3-butanediol production with a volumetric and specific productivity, underlining its potential for microbial chemical production. Although titer and yield of 2,3-butanediol synthesis from glucose were lower compared to other hosts such as *E. coli*, exceptionally high substrate turnover and product formation rates were obtained. Based on the excellent starting point provided here, strain and process optimization could transform *V. natriegens* into a superior 2,3-butanediol production host. These findings also underline the potential of *V. natriegens* as novel host for microbial chemical production in general.

CRediT authorship contribution statement

Anna Maria Erian:Investigation, Data curation, Methodology, Writing - original draft, Writing - review & editing.**Philipp Freitag**:Investigation, Data curation, Writing - review & editing.**Martin Gibisch**:Investigation, Writing - review & editing.**Stefan Pflügl**:Data curation, Methodology, Writing - original draft, Writing - review & editing.

Table 2
Comparison of 2,3-butanediol production in *Vibrio natriegens* with recombinant *Escherichia coli*. The results of this study are given as the mean of two independent biological replicates with the standard deviation as error. Y_{diol}: sum of diols [g] per substrate [g], r: volumetric rates given as component [g] per reaction volume [L] and time [h], q: specific rates given as component [g] per biomass [g] and time [h].

Organism	Genetic construct[a]	Cultivation mode	Medium and carbon source	2,3-Butanediol [g L^{-1}]	Acetoin [g L^{-1}]	Y_{diol} [g g^{-1}]	r_{diol} [g L^{-1} h^{-1}]	q_{diol} [g g^{-1} h^{-1}]	r_S [g L^{-1} h^{-1}]	q_S [g g^{-1} h^{-1}]	Source
E. coli BL21(DE3)	pET-RABC	Pulsed fed-batch	Complex, glucose	73.8	9.34	0.46	1.34	n.a.	n.a.	n.a.	Xu et al. (2014)
E. coli W Δ*ldhA* Δ*pflB* Δ*adhE* Δ*lpdA*::K. lpd (E354K) Δ*mdh* Δ*arcAgltA*(R164L)	pSTVM2	Pulsed fed-batch	Complex, glucose	88.0	0	0.35	1.87	n.a.	n.a.	n.a.	Hwang et al. (2018)
E. coli W Δ*ldhA* Δ*adhE* Δ*pta* Δ*frdA*	p445_Ediss	Pulsed fed-batch	Defined, glucose	68.12 ± 1.08	0.13 ± 0.06	0.38 ± 0.01	1.32 ± 0.21	0.15 ± 0.03	3.51 ± 0.57	0.40 ± 0.08	Erian et al. (2018)
E. coli W	p445_Ediss	Pulsed fed-batch	Defined, sugar beet molasses	56.3 ± 2.0	0.00	0.44 ± 0.03	1.31 ± 0.06	0.16 ± 0.01	3.01 ± 0.22	0.38 ± 0.02	Erian et al. (2018)
V. natriegens DSM759	p445_Ediss	Pulsed fed-batch	Complex, glucose	36.39 ± 3.68	10.42 ± 0.67	0.22 ± 0.01	3.88 ± 0.93	0.27 ± 0.03	20.03 ± 0.51	1.44 ± 0.23	This study
V. natriegens DSM759	p445_Ediss	Pulsed fed-batch	Defined, glucose	15.89	11.54	0.22	2.67	0.28	12.24	1.26	This study
V. natriegens DSM759	p445_Ediss	Chemostat	Defined, glucose	5.09 ± 0.34	1.44 ± 0.19	0.35 ± 0.01	2.04 ± 0.04	0.66 ± 0.01	5.8 ± 0	1.88 ± 0.07	This study
V. natriegens DSM759	p445_Ediss	Chemostat	Defined, sugar beet molasses	4.66 ± 0	0.15 ± 0.12	0.24 ± 0.01	1.5 ± 0.04	0.3 ± 0.02	6.18 ± 0	1.23 ± 0.04	This study

n.a. not available.
It is noted that the pulsed fed-batch cultivations without yeast extract showed a high degree of variability likely connected to a sensitivity to glucose starvation observed for *V. natriegens*. Therefore, only one cultivation is shown as an example here due to difficulties in reproducibility.

[a] pET-RABC contained *budB*, *budA* and *budC* under control of *bud* operon promoter of *Enterobacter cloacae* subsp. *dissolvens* SDM. pSTVM2 contained *aldB* under control of a weak and *ilvBN* and *bdh1* under control of a strong *nar* promoter. p445_Ediss contained promoters from the Anderson promoter library and genes from *E. cloacae* subsp. *dissolvens* DSM16657: *budB* and *budA* under control of promoter 23114 and *budC* under control of promoter 23105.

Table 3
Chemostat cultivations of *V. natriegens* 445_Ediss using glucose and sucrose from sugar beet molasses at different oxygen conditions. Cultivations were carried out using a dilution rate of 0.313 h^{-1}, T = 37 °C, pH 6.5 and a stirrer speed of 1000 rpm. Gassing rate and gas composition were as indicated. Results are from two independent biological replicates and standard deviations are given as errors. $Y_{X/S}$: sum of biomass [g] per substrate [g], $Y_{diol/S}$: sum of 2,3-butanediol and acetoin [g] per substrate [g], $Y_{by\text{-}product/S}$: sum of acetic, succinic, formic, lactic and pyruvic acid and ethanol [g] per substrate [g], $Y_{CO2/S}$: sum of CO_2 [mol] per substrate [mol], r: volumetric rates given as component [g]/[mmol] per reaction volume [L] and time [h], q: specific rates given as component [g]/[mmol] per biomass [g] and time [h].

	Glucose	Glucose	Sugar beet molasses
Concentration [g L^{-1}]	18.20	18.53	19.75
Gassing rate [vvm]	0.25	1.00	1.00
Oxygen [%]	21	5	5
Biomass [g L^{-1}]	7.36 ± 0.39	3.09 ± 0.11	5.04 ± 0.17
2,3-Butanediol [g L^{-1}]	3.09 ± 0.11	5.09 ± 0.34	4.66 ± 0.01
Acetoin [g L^{-1}]	0.54 ± 0.18	1.44 ± 0.19	0.15 ± 0.12
Diol ratio	1.98 ± 0.64	3.89 ± 0.88	29.97 ± 13.64
Acetic acid [g L^{-1}]	0 ± 0	0.69 ± 0.02	1.26 ± 0.15
Succinic acid [g L^{-1}]	0.01 ± 0.02	0.26 ± 0.06	0.94 ± 0.21
Formic acid [g L^{-1}]	0.03 ± 0.05	0.14 ± 0.10	0.55 ± 0.07
Lactic acid [g L^{-1}]	0 ± 0	0.10 ± 0.05	0.77 ± 0.05
Ethanol [g L^{-1}]	0 ± 0	0.33 ± 0.14	0 ± 0
Pyruvic acid [g L^{-1}]	0 ± 0	0 ± 0	0.39 ± 0.04
r_S [g L^{-1} h^{-1}]	5.70 ± 0.01	5.80 ± 0.01	6.18 ± 0.01
q_S [g g^{-1} h^{-1}]	0.78 ± 0.04	1.88 ± 0.07	1.23 ± 0.04
r_{diol} [g L^{-1} h^{-1}]	0.56 ± 0.04	2.04 ± 0.04	1.50 ± 0.04
q_{diol} [g g^{-1} h^{-1}]	0.08 ± 0.01	0.66 ± 0.01	0.30 ± 0.02
r_{CO2} [mmol L^{-1} h^{-1}]	85.66 ± 2.80	60.43 ± 1.26	56.53 ± 8.98
q_{CO2} [mmol g^{-1} h^{-1}]	11.65 ± 0.24	19.60 ± 0.32	11.29 ± 2.15
r_{O2} [mmol L^{-1} h^{-1}]	29.12 ± 3.01	29.46 ± 0.75	23.61 ± 1.15
q_{O2} [mmol g^{-1} h^{-1}]	3.97 ± 0.62	9.56 ± 0.59	4.70 ± 0.38
$Y_{X/S}$ [g g^{-1}]	0.40 ± 0.02	0.17 ± 0.01	0.26 ± 0.01
$Y_{diol/S}$ [g g^{-1}]	0.10 ± 0.01	0.35 ± 0.01	0.24 ± 0.01
$Y_{by\text{-}product/S}$ [g g^{-1}]	0 ± 0	0.08 ± 0.02	0.13 ± 0.02
$Y_{CO2/S}$ [mol mol^{-1}]	2.58 ± 0.1	1.79 ± 0.09	3.03 ± 0.27
C-recovery [%]	102.0 ± 1.6	100.3 ± 0.1	103.2 ± 0.2

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by the Austrian Research Promotion Agency [grant number 858702].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2020.100408.

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