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Utilization of acid whey and oat pomace in succinic acid fermentation

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ABSTRACT

Aim: of this study was to investigate the by-products acid whey and oat pomace as nutrient sources for succinic acid production by *Actinobacillus succinogenes*. Both by-products provide carbon sources in form of glucose and/or lactose without any pre-treatment. Yields of succinic acid per g total sugars consumed after 24 h were between 0.6 and 0.7 in control medium, acid whey, and in acid whey/oat pomace mixtures. A yield of more than 0.8 g per g was found in oat pomace after 24 h, which further increased to 1.0 g per g after 48 h. For the fermentation carried out with acid whey and oat pomace mixed at a ratio of 1:1 a productivity of 0.52 g L^{-1} h^{-1} was obtained. The productivities in control medium, acid whey, oat pomace, acid whey/oat pomace (2:1), and acid whey/oat pomace (3:1) were 16 %, 75 %, 48 %, 46 %, and 48 % less, respectively, indicating the necessity of finding the right balance of nutrients. The results of this study contribute to the decentralized utilization of food residues and even, despite the high value of succinic acid as platform chemical, to a recirculation into new food products.

1. Introduction

Succinic acid, a 4-carbon dicarboxylic acid, has a wide range of uses in polyesters, paints, fuel additives, herbicides, medicines, and detergents, making it one of the top platform chemicals derived from biomass. The need for bio-based succinic acid is being driven by the pressing need to find green and renewable chemicals, which can be produced at reduced carbon footprint, cost effective, at less volatile prices, and lessen reliance on crude oil [12].

Bio-based succinic acid can be produced from organic residues through microbial fermentation [14]. For the production of bio-based succinic acid, a variety of microorganisms have been employed, including *Actinobacillus succinogenes, Mannheimia succiniciproducens, Pichia kudriavzevii, Saccharomyces cerevisiae, Corynebacterium glutamicum, Basfia succiniciproducens*, and *Escherichia coli*. [4]. Additionally, various organic substrates have been employed as carbon sources for the manufacture of succinic acid, such as glycerol, frying oil from sunflower waste, agro-industrial waste, and hydrolysates from the organic part of municipal solid waste [16,20,9]. It has been shown that succinic acid yields and titers can vary depending on the applied feedstocks. Using pure glycerol a maximum titer of 117 g L^{-1} and a yield of 1.3 g g^{-1} could be obtained using a strain of the class Actinobacteria [6]. When corncob hydrolysate was used directly as carbon source, a titer of 23.64 g L^{-1} and a yield of 0.58 g g^{-1} was reached [23]. Succinic acid production from hydrolysates of municipal solid waste resulted in a titer of 29.4 g L^{-1} in batch cultures and 21.2 g L^{-1} in continuous cultures [16]. Terboven et al. made use of a lactose concentrate appearing as by-product from cheese-making as substrate for the *A. succinogenes* or *B. succiniciproducens*. An initial sugar concentration of 43 g L^{-1} and 5 g L^{-1} of yeast extract resulted in a comparable performance of both strains. The best yield and acid titer were 0.57 g g^{-1} and 23 g L^{-1}. The authors have further shown that an appropriate supply of yeast extract is important to achieve a proper succinic acid production [18].

From the examples shown, it can be concluded that high titers can only be achieved using substrates that do not only contain utilizable carbon sources but also nitrogen sources. Thus, the right feedstocks must be chosen to establish a commercially feasible bio-based succinic acid production process. Economically speaking, low-cost raw materials like agricultural or food leftovers should be used for the commercial bio-based synthesis of succinic acid, which ideally do not require an intensive upstreaming to make nutrients available [13]. Furthermore, the feedstocks and the selected microorganism shall allow for the production of succinic acid at a high titer and productivities as well as minimized side products formation (predominantly organic acids) to result in

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Table 1
Composition of the different batches (OP1 and OP2) of oat pomace (DM: Dry matter; oDM: Organic dry matter).

Parameter	OP1	OP2
DM [%, w w^{-1}]	23.5	32.1
oDM [% DM, w w^{-1}]	94.4	92.1
Proteins [% DM, w w^{-1}]	23.0	37.5
Sugars [% DM, w w^{-1}]	26.5	5.9
Starch [% DM, w w^{-1}]	4.1	16.4
Hemicellulose [% DM, w w^{-1}]	18.5	16.9
Cellulose [% DM, w w^{-1}]	3.2	3.7
Lignin [% DM, w w^{-1}]	4.2	6.1

a pure succinic acid [4].

Finding the right balance between upstreaming and fermentation was the aim of the present study. Based on the two by-products, acid whey and oat pomace, appropriate upstreaming was developed which resulted in most efficient conversion of sugars into succinic acid by *A. succinogenes*. In the present study both residues were used as nitrogen and carbon sources with a focus on substituting yeast extract but still to supply sufficient nitrogen for a high titer [18]. Since both by-products are locally generated by food producers, the presented approach should be considered as process for the decentralized utilization, and thus the obtained results strongly support the establishment of a local bioeconomy.

2. Material and methods

2.1. Strain

A. succinogenes (DSM 22257) was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and used in all fermentations. Prior to fermentation the strain was reactivated with two cryo-beads in 35 mL tryptone soy bouillon (TSB) consisting of 17 g L^{-1} peptone from casein, 3 g L^{-1} peptone from soy, 2.5 g L^{-1} K_2HPO_4, 5 g L^{-1} NaCl, and 2.5 g L^{-1} glucose monohydrate at 37 °C for 20 h in an incubator.

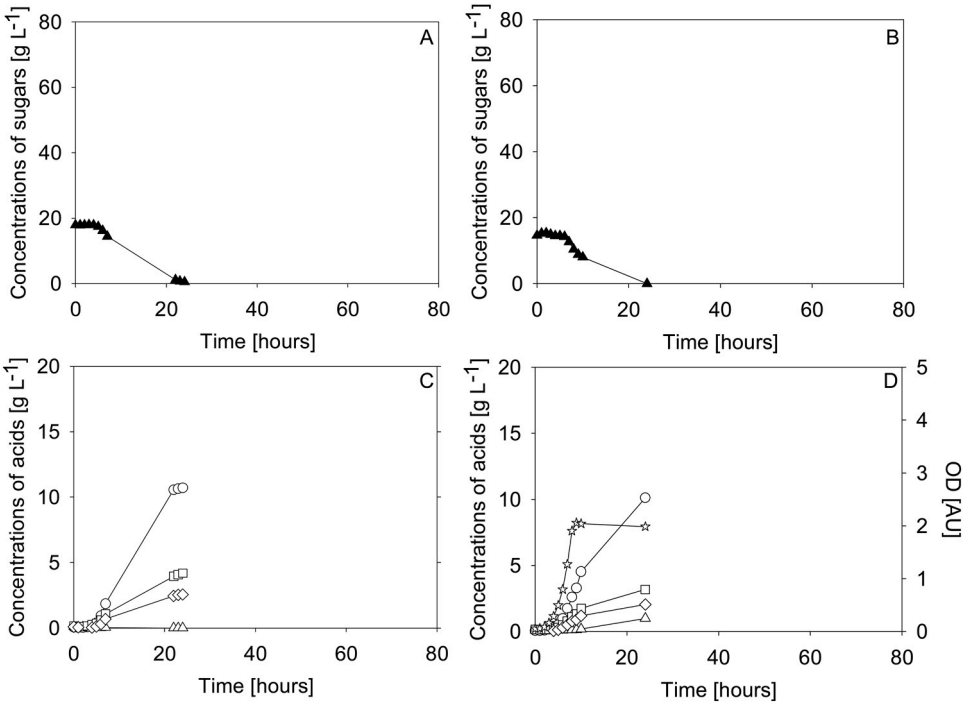

Fig. 1. Two fermentations of *Actinobacillus succinogenes* in filtrated control medium. A and B: Consumption of glucose (closed triangle), C and D: Formation of succinic acid (open circled), lactic acid (open triangle), acetic acid (open quadrate), and formic acid (open diamond) as well as increase in optical density (OD, open star). CO_2 was used as additional carbon source.

Table 2
Effect of different substrates on the production performance of succinic acid and the formation of acetic and formic acids by *Actinobacillus succinogenes* after 24 and 48 h (Y_{SA}: Yield of succinic acid per g sugar; P_{SA}: Succinic acid productivity; SA: Succinic acid; AA: Acetic acid; FA: Formic acid).

Substrate	Y_{SA} [g/g]		P_{SA} [g L^{-1} h^{-1}]		SA [g L^{-1}]		Ratio of SA to AA to FA	
	24 h	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
Control medium	0.61	-	0.44	-	10.66	-	1:0.38:0.23	-
	0.69	-	0.42	-	10.06	-	1:0.30:0.20	-
Acid whey	0.62	0.95	0.18	0.18	4.42	8.72	1:0.41:0.31	1:0.28:0.21
Suspended oat pomace (OP1)	0.84	-	0.26	-	6.18	-	1:0.31:0.31	-
	0.80	1.00	0.27	0.28	6.37	10.88	1:0.28:0.32	1:0.19:0.21
Acid whey/suspended oat pomace (ratio 1:1, OP1)	0.68	0.58	0.52	0.36	12.63	18.40	1:0.40:0.32	1:0.33:0.26
Acid whey/suspended oat pomace (ratio 2:1, OP2)	0.63	0.77	0.25	0.29	5.92	13.69	1:0.33:0.26	1:0.26:0.24
	0.69	0.62	0.28	0.23	6.81	10.86	1:0.25:0.23	1:0.51:0.23
Acid whey/suspended oat pomace (ratio 3:1, OP2)	0.66	0.59	0.26	0.28	6.10	13.60	1:0.28:0.09	1:0.23:0.12
	0.68	0.66	0.27	0.28	6.52	13.43	1:0.23:0.16	1:0.36:0.06

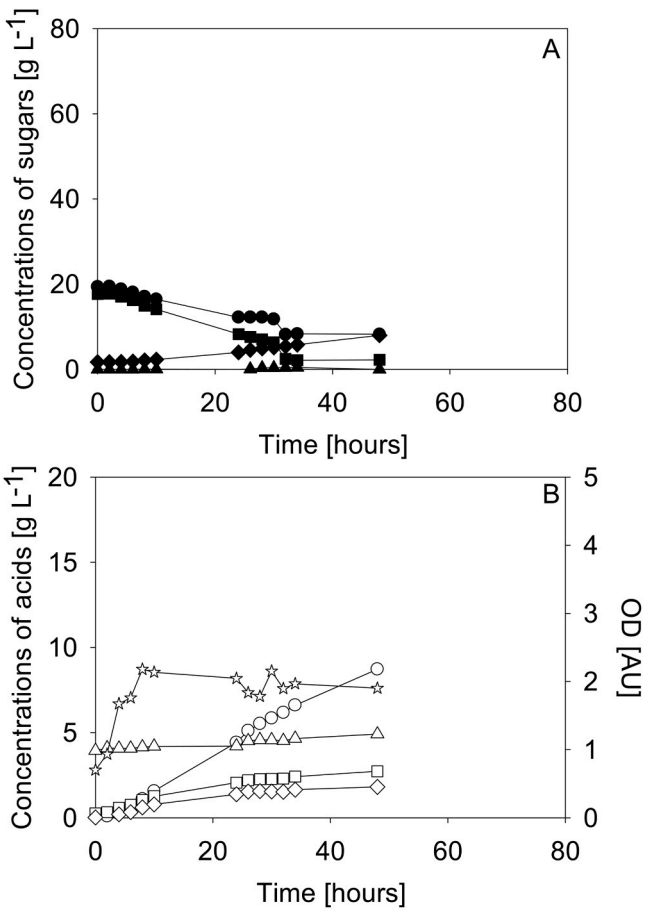

Fig. 2. Fermentation of *Actinobacillus succinogenes* in filtrated acid whey. A: Concentrations of lactose (closed quadrate), galactose (closed diamond), and total sugars (closed circle), B: Formation of succinic acid (open circled), lactic acid (open triangle), acetic acid (open quadrate), and formic acid (open diamond) as well as increase in optical density (OD, open star). CO_2 was used as additional carbon source. Please note that the fermentation was carried out only once.

2.2. By-products

Acid whey is a by-product from the manufacture of low-fat curd production. To produce the low-fat curd, the raw milk was separated, and the resulting low-fat milk pasteurized and heated. After cooling, the milk was fermented by adding lactic acid bacteria and rennet. The solid components of the curdled milk were separated from the liquid components (acid whey) using separators.

Oat pomace is a by-product of oat milk production. The process of making oat milk involved soaking oats and treating them with enzyme [3]. Afterwards oat milk was decanted from the solids (oat pomace). In this study, two batches of oat pomace (OP1 and OP2), obtained through different process steps during oat milk production, with different compositions have been used. OP1 pomace resulted from oat flakes that were mixed with water, whereas OP2 pomace were derived from oat flakes mixed with water and grounded at the same time. The composition of both is shown in Section 3.

Both by-products were collected regularly from local producers and immediately frozen at −10 °C until used in experiments.

2.3. Preparation of fermentation media

The by-products acid whey and oat pomace were used for preparing different fermentation media. Acid whey and oat pomace were either purely used in fermentation or mixed in proportions 1:1 (v/w), 2:1 (v/w) or 3:1 (v/w) for identifying the right balance of carbon and nitrogen sources to achieve high yield and titers under consideration that the suspension should be pumpable for subsequent processing. Acid whey, oat pomace, and mixtures of acid whey and oat pomace were stirred magnetically at room temperature for 1 h with a rate of 300 rpm. The media was divided into four large centrifuge tubes and centrifuged for 10 min at 14.025*g* (Rotanta 460R, Hettich GmbH & Co. KG, Germany). The supernatants of suspended oat pomace or mixtures as well as pure acid whey were filtered using a pleated filter (320 mm × 13 µM, ROTILABO® Typ 600P, Roth, Germany) and used in fermentations.

2.4. Fermentation

Succinic acid fermentation was carried out in 1 L EloFerm bioreactors (EloSystems GmbH, Berlin, Germany) with a working volume of 300 mL under controlled conditions (37 °C and pH 6.7) and stirred at 30 rpm using a magnetic bar. The pH was adjusted with 5 M NaOH. CO_2 was supplied by dosing either a 20 % (w/v) Na_2CO_3 solution, coupled with pH control, or by dosing pure CO_2 at 0.2 mL per minute. The bioreactor was sterilized at 110 °C for 10 min together with the substrate, which was either suspended oat pomace, acid whey, a mixture of both, or control medium consisting of 15 g L^{-1} glucose, 5 g L^{-1} yeast extract, and 1 g L^{-1} $NaHCO_3$. To all media 8 drops of antifoam agent (Antifoam A concentrate, Sigma, Germany) were added. The fermentation medium was inoculated with pre-cultured *A. succinogenes* in TSB-medium. Control medium consisted of 15 g L^{-1} glucose, 5 g L^{-1} yeast extract, and 1 g L^{-1} $NaHCO_3$. In all fermentations which corresponded to an optical density (OD) of 0.1 (in control medium). Fermentations were carried out in duplicate if not otherwise stated in Section 3.

For the measurement of OD, sugars, and organic acids concentrations, samples were taken regularly. Samples for sugars and succinic acid concentration determination were centrifuged at 13,400*g* for 10 min and kept frozen until analysis.

2.5. Analytics

OD was determined at 660 nm using a Cary 6000i UV–VIS–NIR spectrometer (Agilent).

To determine the dry matter of applied residues a halogen moisture analyzer (HR 83, Mettler Toledo, Germany) was used.

The ash content and eventually the organic matter were determined by treating a defined portion of a sample at 550 °C for 5 h in a muffle furnace.

The ANKOM2000 fiber analyzer (ANKOM Technology, USA) was employed to measure the fiber composition of oat pomace, in particular the cellulose, hemicellulose, and lignin contents according to the Weender analysis. Firstly, samples were pre-dried at 60 °C and afterwards cellulose, hemicellulose, and lignin contents were measured following manufacture's protocol (ANKOM Technology).

Total nitrogen was quantified as Kjeldahl nitrogen. All chemical forms of nitrogen in the sample are determined as the sum of the organically bound nitrogen, ammonium-nitrogen, and nitrate-nitrogen. The distillation was carried out with the Kjeldahl Sampler System K-370/371. The amount of proteins was calculated from the amount of Kjeldahl nitrogen multiplied by a conversion factor of 6.25.

Starch was determined in oat pomace in accordance with the international standard ISO 10520.

Free sugars in oat pomace were determined after resuspending the material in water and analyzing the supernatant with the HPLC-method described below.

Free amino nitrogen (FAN) was determined following the modified EBC-ninhydrin method. First, two reagents were prepared. For reagent A, 1 g Na_2HPO_4*$12H_2O$, 0.6 g KH_2PO_4, 0.05 g ninhydrin, and 0.03 g fructose were dissolved in 10 mL demineralized water. Reagent B contained 0.2 g KIO_3, 60 mL demineralized water, and 40 mL absolute ethanol. For analysis, 20 µL sample, 50 µL A, and 30 µL demineralized

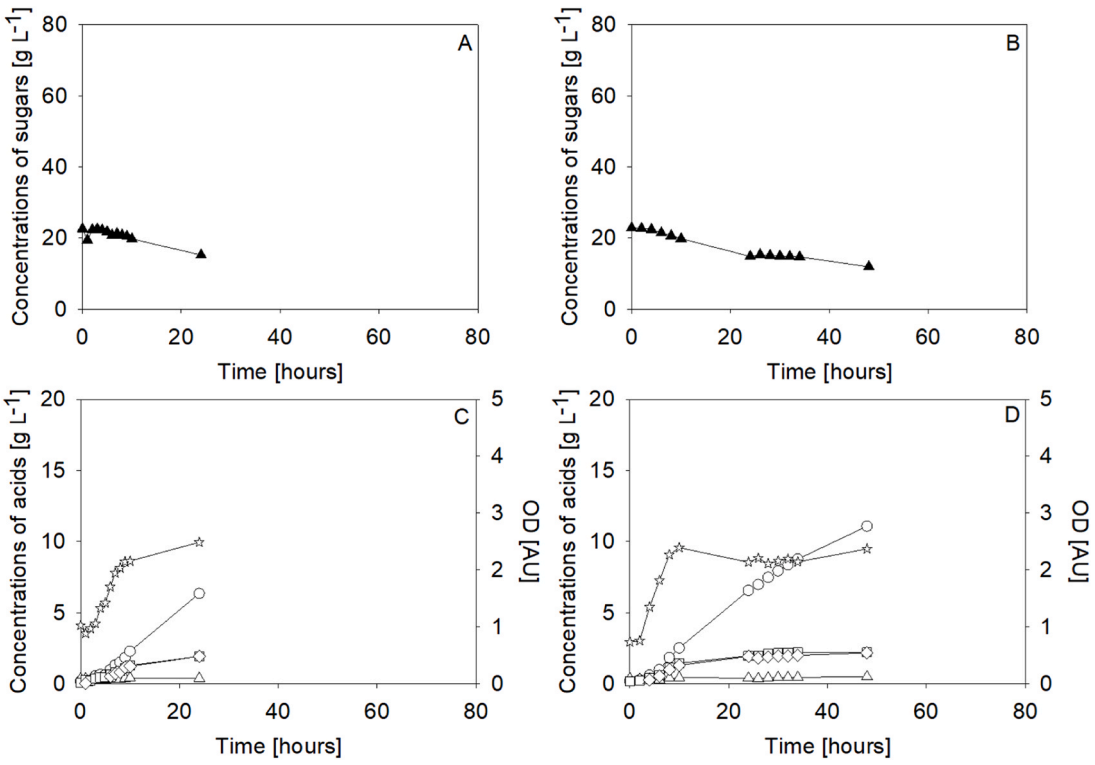

Fig. 3. Two fermentations of *Actinobacillus succinogenes* in filtrated suspended oat pomace (OP1). A and B: Concentrations of glucose (closed triangle), lactose (closed quadrate), galactose (closed diamond), and total sugars (closed circle), C and D: Formation of succinic acid (open circled), lactic acid (open triangle), acetic acid (open quadrate), and formic acid (open diamond) as well as increase in optical density (OD, open star). CO_2 was used as additional carbon source.

water were combined and heated at 90 °C for 5 min. Then 900 µL of B were added and absorption at 570 nm was measured. A calibration curve with glycine was used as reference.

Sugars as well as organic acids concentrations were determined using HPLC (Thermo Scientific Dionex UltiMate 3000, Germany): 10 µL of sample was injected on an Aminex HPX-87H column (300 mm × 7.8 mm) and eluted isocratically with 0.4 mL min^{-1} 5 mM H_2SO_4 at 50 °C. Calibration curves were generated with pure solutions of known concentration.

3. Results

3.1. Composition of by-products

The composition of the two by-products differed. Acid whey was liquid containing lactose, galactose, lactic acid, and total nitrogen with concentration of 38.2, 2.8, 6.5, and 1.1 g L^{-1}, respectively. Both tested oat pomaces had a water content between 69 % and 76 % (w/w) and an organic content of more than 90 %/w/w). For fermentation purposes the value of oat pomace lays in its protein and carbohydrate contents (Table 1). Interestingly, OP1 differed in terms of protein, sugars, and starch contents from OP2. OP1 contained with 23 % (w/w) only 2/3 of the proteins found in OP2. However, OP1 contained with 26.5 % (w/w) 4-times more free sugars than OP2. Contrarily, OP1 contained 4-times less starch than OP2. The contents for hemicellulose, cellulose, and lignin were rather comparable between both oat pomaces (Table 1). Since oat pomace has not been hydrolyzed to make all carbohydrates available, a conversion of hemicellulose, cellulose, and lignin into succinic acid can be ruled out.

Even though there were variations in the biochemical composition of oat pomaces, which probably resulted from different processing steps and the use of different oat plant species, the presence of proteins, sugars, and starch makes it a promising nutrient source for fermentation. Mixing oat pomace in water or alternatively acid whey at room temperature for 1 h was already sufficient to extract nutrients. This simplified the extraction process and saves cost compared to an enzyme-based nutrient recovery process during upstreaming.

3.2. Fermentation in control medium, acid whey, or suspended oat pomace

Succinic acid fermentation was investigated in pure acid whey and suspended oat pomace, and the results compared to a control medium, which consisted of 15 g L^{-1} glucose, 5 g L^{-1} yeast extract, and 1 g L^{-1} $NaHCO_3$, in terms of titer, yield, and productivity. As visible in Fig. 1 A to D, glucose was totally consumed within 24 h and more than 10 g L^{-1} was produced in both fermentations. Together with succinic acid, acetic, and formic acids were produced, while lactic acid was in the medium from the beginning and not formed by *A. succinogenes*. The concentrations of the three acids were 3.2 g L^{-1}, 2.0 g L^{-1}, and 1.0 g L^{-1}, respectively. The ratio of acetic and formic acids compared to succinic acid was 1:0.30:0.20. In average a yield of 0.64 g per g glucose and a productivity of 0.43 g L^{-1} h^{-1} were achieved (Table 2).

The fermentation in pure acid whey gave a different result compared to the fermentation in the control medium. Lactose was with 17.7 g L^{-1} the dominant sugar (Fig. 2A). Additionally, 1.7 g L^{-1} galactose was present. Within 24 h, 7 g L^{-1} of the total sugars were consumed and 4.4 g L^{-1} succinic acid, 1.7 g L^{-1} acetic acid, 1.3 g L^{-1} formic acid, and 0.3 g L^{-1} lactic acid were found (Fig. 2B). Eventually the yield of succinic acid per g consumed total sugar was 0.62 and the productivity was 0.18 g L^{-1} h^{-1}. Titer and productivity were less than half compared to what has been found in the control (Table 2). Lactose concentration was reduced, however, galactose resulting from the cleavage of lactose was not utilized by *A. succinogenes*. Per g of lactose that was reduced, 0.3 g of galactose was formed. By extending the fermentation period to 48 h lactose concentration was further reduced to 2.2 g L^{-1} and in total 8.0 g L^{-1} galactose was determined in the fermentation broth. Nevertheless, succinic acid titer and yield increased to 8.7 g L^{-1} and

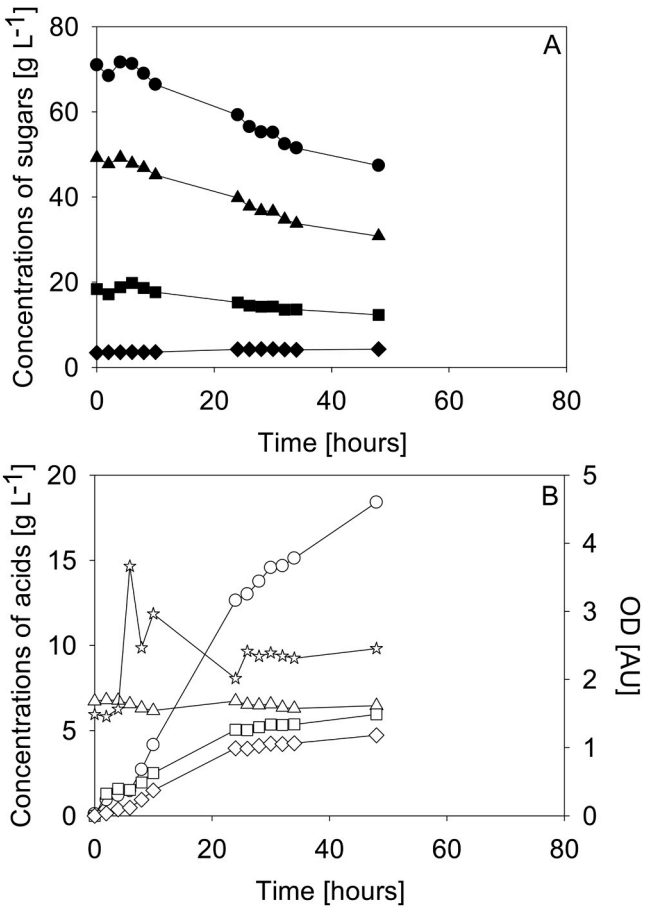

Fig. 4. Fermentation of *Actinobacillus succinogenes* in acid and suspended oat pomace (OP2) mixed in a ratio of 1:1. A: Concentrations of glucose (closed triangle), lactose (closed quadrate), galactose (closed diamond), and total sugars (closed circle), B: Formation of succinic acid (open circled), lactic acid (open triangle), acetic acid (open quadrate), and formic acid (open diamond) as well as increase in optical density (OD, open star). CO_2 was used as additional carbon source. Please note that the fermentation was carried out only once.

0.95 g L^{-1} h^{-1}. While productivity was comparable to what has been found after 24 h (Table 2).

Furthermore, two fermentations were carried out with pure suspended oat pomace (OP1, Fig. 3A–D). Glucose was the only sugar present at around 22 g L^{-1} (Fig. 3 A and B). After 24 h, both fermentations resulted in 6.3 g L^{-1} succinic acid, a yield of 0.82 g g^{-1}, and a productivity of 0.26 g L^{-1} h^{-1} (Table 2). Simultaneously, 1.9 g L^{-1} of acetic acid and formic acid were found. Lactic acid was not found in oat pomace suspension (Fig. 3 C and D). The ratio of succinic acid to acetic acid to formic acid was 1:0.29:0.31 (Table 2). One fermentation was further extended to 48 h (Fig. 3 B and D). No further decrease in glucose could be determined, however, succinic acid concentration increased to nearly 11 g L^{-1}, while the other acids did not increase in concentrations. The increased succinic acid concentration resulted in a yield and productivity of 1.0 g g^{-1} and 0.28 g L^{-1} h^{-1}. The ratio of succinic acid to acetic acid to formic acid after 48 h was 1:0.19:0.21 (Table 2).

3.3. Fermentation in mixtures of acid whey and suspended oat pomace

In the following it was investigated to which ratio acid whey and suspended oat pomace should be mixed to increase the titer, yield, and productivity of succinic acid. For this purpose, a 1:1 mixture was prepared with 700 g of oat pomace (OP2), made up to a volume of 1.4 L with acid whey. A maximum sugar concentration of 71 g L^{-1} was examined as a fermentation medium, which was sterile-filtered and gassed using CO_2. Fig. 4 shows the fermentation with 71 g L^{-1} total sugar. After 24 h, 12.6 g L^{-1} succinic acid was formed, corresponding to a yield of 0.68 g g^{-1} total sugars and a volumetric productivity of 0.52 g L^{-1} h^{-1}. After 48 h a concentration of 18.4 g L^{-1} succinic acid could be achieved. However, only 33.3 % of the total sugar was consumed, leaving 47.3 g L^{-1} unused in the medium. The concentration of acetic acid, formic acid, and lactic acid after 48 h was 6.0 g L^{-1}, 4.7 g L^{-1}, and 6.5 g L^{-1}. Also in this fermentation lactic acid was present from the beginning and not formed by *A. succinogenes*. The ratio of the acids formed from succinic acid to acetic acid to formic acid was 1:0.33:0.26 (Table 2).

Based on the obtained results a substrate mixture with an initial total sugar concentration between 20 and 70 g L^{-1} was selected for further experiments. For this purpose, acid whey and oat pomace (OP2) were mixed in a ratio of 2:1 and then sterilized using an autoclave. Overall, a total sugar concentration of around 35 g L^{-1} was achieved (Fig. 5 A-D). From both fermentations in average 12.3 g L^{-1} succinic acid was obtained after 48 h and a yield of 0.70 g g^{-1} total sugars and a volumetric productivity of 0.26 g L^{-1} h^{-1} were obtained. The by-products formed reached a ratio of succinic acid to acetic acid to formic acid of 1:0.26:0.24 (Fig. 5A and C, Table 2) and 1:0.51:0.23 (Fig. 5 B and D, Table 2).

In subsequently carried out fermentations the ratio of acid whey to oat pomace (OP3) was increased to 3:1 (Fig. 6 A-D). Both carried out fermentations revealed similar results and 13.4 g L^{-1} of succinic acid could be produced after 48 h and a yield of 0.66 g g^{-1} and a volumetric productivity of 0.28 g L^{-1} h^{-1} achieved (Table 2). After 48 h, 45 % of the initial total sugar supplied remained. To further improve the utilization of sugars the fermentation length was increased to nearly 80 h (Fig. 6 A and C). Increasing the fermentation length resulted in a further consumption of total sugars but still 20 % remained in the medium. The succinic acid titer increased to 17 g L^{-1}. Interestingly, the other acetic and formic acids did not further increase in concentrations.

4. Discussion

Establishing a fermentative succinic acid production depends on the availability of substrates and their quality. In this study the by-products acid whey and oat pomace have been investigated as nutrient sources for *A. succinogenes* to produce bio-based succinic acid Oat is known for its nutritional quality resulting from a high-quality protein, unsaturated fatty acids, and soluble fiber (ß-glucan) contents as well as the presence of polyphenolic compounds and micronutrients [1]. The composition of oat grains, however, can vary between countries where it is grown [1] and varieties [15]. Furthermore, the manufacturing process during the oat drink production could influence the composition of the resulting oat pomace. In 2017, oat ranked with 25.9 million tons seventh of the produced grains worldwide [8] with increasing tendency. With the increasing popularity of oat drinks, it is expected that the appearance of oat pomace will increase. Furthermore, more than 180 million tons of acid whey are produced globally per year. It can, for instance, be used for the formation of whey powder after concentration, however, this might be due to a high content of minerals not applicable to acid whey. Furthermore, smaller scale cheese making facilities may not have the equipment to concentrate whey, and thus use it as feed for animals [2]. It can be expected that a focus on oat pomace and acid whey as substrates for the fermentative production of succinic acid is justified and a continuous supply of substrates can be secured. The focus is further justified by the promising composition in terms of sugars (glucose and lactose) and nitrogen compounds as shown in Table 1. That whey is a promising substrate for *A. succinogenes* and for the formation of succinic acid has been shown in a couple of studies before [10,19,21,7]. The feasibility of oat pomace, however, was tested only once [5] and more knowledge on mixing ratios of acid whey and oat pomace is needed.

In all fermentations, irrespective if carried out with control medium, pure suspended oat pomace, acid whey, or mixtures of oat pomace and

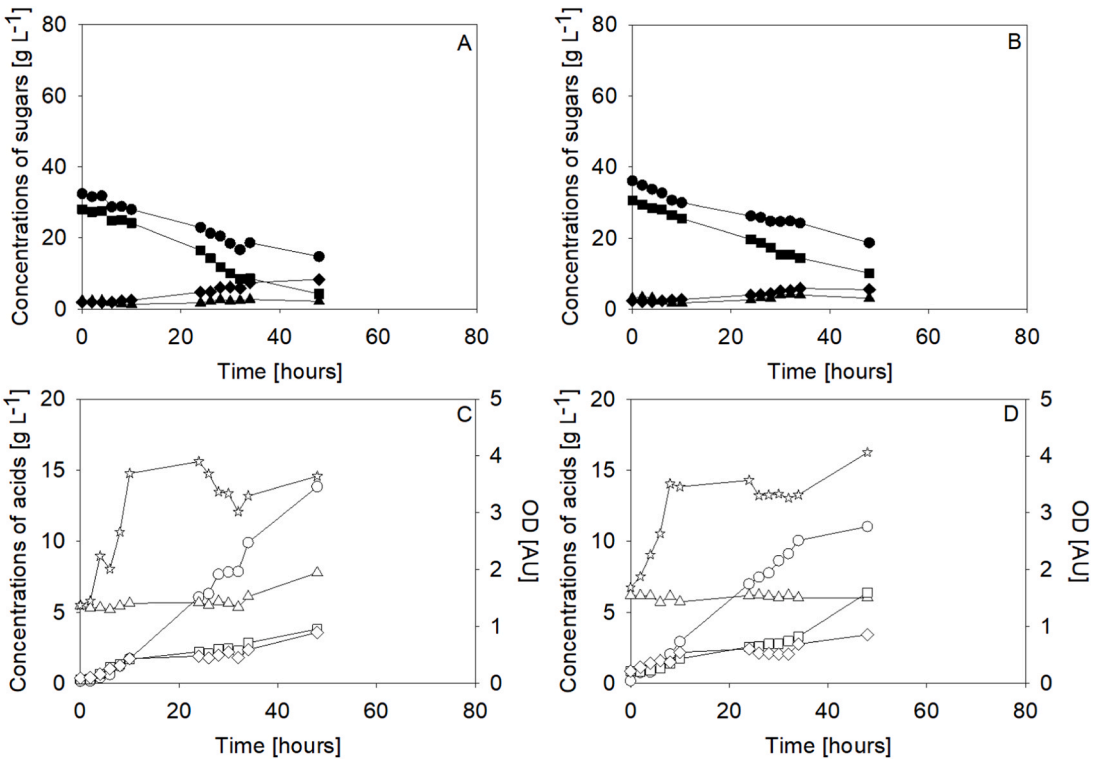

Fig. 5. Two fermentations of *Actinobacillus succinogenes* in autoclaved acid whey and suspended oat pomace (OP2) mixed in a ratio of 2:1. A and B: Concentrations of glucose (closed triangle), lactose (closed quadrate), galactose (closed diamond), and total sugars (closed circle), C and D: Formation of succinic acid (open circled), lactic acid (open triangle), acetic acid (open quadrate), and formic acid (open diamond) as well as increase in optical density (OD, open star). For the fermentation shown in A and B Na_2CO_3 and for the one shown in C and D CO_2 were used as additional carbon source.

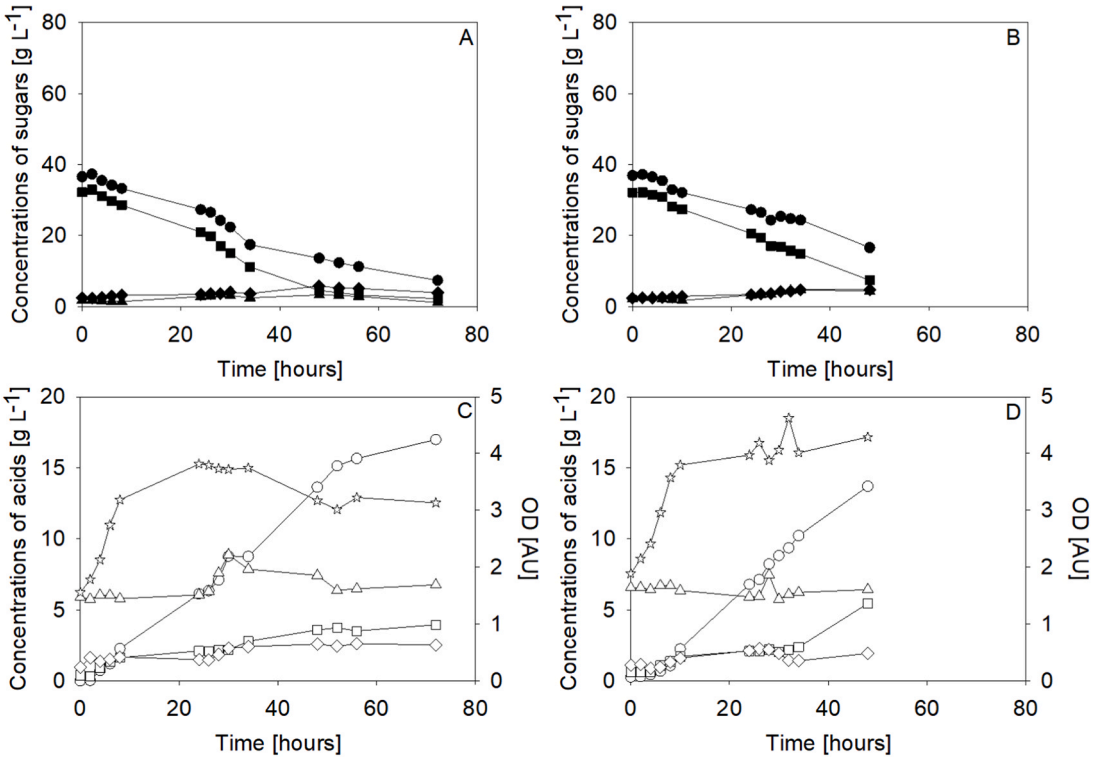

Fig. 6. Two fermentations of *Actinobacillus succinogenes* in autoclaved acid whey and suspended oat pomace (OP2) mixed in a ratio of 3:1. A and B: Concentrations of glucose (closed triangle), lactose (closed quadrate), galactose (closed diamond), and total sugars (closed circle), C and D: Formation of succinic acid (open circled), lactic acid (open triangle), acetic acid (open quadrate), and formic acid (open diamond) as well as increase in optical density (OD, open star). Na_2CO_3 was used as additional carbon source.

acid whey, the OD increased during the first 8–9 h (Figs. 1–6). This was also the time where a decrease in the concentration of FAN from roughly 0.1 g L^{-1} to 0.08 g L^{-1} could be measured (not shown). It is interesting that the formation of succinic acid is not associated to the growth of *A. succinogenes* as in most fermentations succinic acid concentration increased when OD stagnated. To ease the downstream processing of succinic acid the formation of side products such as acetic and formic acids should be avoided. Unfortunately, it can be seen from the results obtained that acetic and formic acids also do increase when growth stagnated and even worst ratios of succinic acid to side products have been found when the fermentation time was extended to 48 h. Additionally, an effect of CO_2 or Na_2CO_3, which have been used as additional carbon sources in the present study, on the formation of side products can be ruled out as comparable results where obtained (Figs. 5 and 6, and Table 2).

While the focus was in the past predominantly on the identification of suitable carbon sources, activities concentrate now on the identification and efficient use of alternative and complex nitrogen sources to produce succinic acid. In various studies, among others, corn steep liquor [22], whey [7,21], and yeast extract [18] have been studied. Terboven et al. found that a supplementation of 5 g L^{-1} yeast extract is more than sufficient when an initial sugar concentration of 43 g L^{-1} is applied [18]. The authors found a titer and a yield of 23 g L^{-1} and 0.57 g per g total sugar. Louasté and Eloutassi found optimal succinic acid production with 25 g L^{-1} lactose in 35 g L^{-1} whey powder, which resulted in a succinic acid titer of 21 g L^{-1} and yield of 0.65 g per g lactose [7]. Xi et al. tested 7.5 g L^{-1} and 15 g L^{-1} of corn steep liquor to substitute yeast extract and obtained 17.1 and 15.1 g L^{-1} succinic acid at a yield of 0.57 and 0.50 g per g glucose. The nitrogen supply has a direct impact on the utilization of carbon sources. While glucose was the preferred carbon source, galactose accumulated in this study. This was also observed before when nitrogen was absent [18]. Interestingly, when nitrogen was supplied in an utilizable form and at sufficient concentration also galactose was utilized [18]. Thus, by providing a better nitrogen source the productivities, as shown in Table 2, can certainly be increased by also making use of the accumulating galactose.

In this study the yield of succinic acid was comparable in control medium, acid whey, and mixtures of oat pomace and acid whey at different ratios (Table 2), and the yields are similar to what has been reported earlier [18,21,22,7]. However, highest yield was obtained in this study in suspended oat pomace. After 24 h the yield was above 0.8 g per g sugar consumed. After 48 h the yield increased to 1.0 g g^{-1} (Table 2 and Fig. 3). It might be speculated that other carbon sources beside glucose were present and utilized by *A. succinogenes*. But also the assimilation of CO_2 might have contributed to a yield of 1 g per g and higher [6,17].

It might be expected that a better pretreatment using proteolytic enzymes may increase the amount of FAN recovered from acid whey and oat pomace. Nevertheless, the upstreaming used in this study is simple and realizable without any special equipment on chemicals. Furthermore, the reduction of process steps may reduce the risks of contamination and may eventually make sterilization unnecessary. Such simplicity may also contribute to the realization of continuous process to increase productivity without complicating upstreaming [11].

5. Conclusions

The use of acid whey and oat pomace as nutrient sources for fermentation has shown promising results, with oat pomace providing a higher succinic acid yield compared to acid whey. The efficient utilization of nitrogen sources is crucial for biomass production and succinic acid formation. However, neither acid whey nor suspended oat pomace provided sufficient nitrogen to allow for an efficient conversion of sugars into succinic acid. Nevertheless, the findings contribute to the efforts to identify suitable carbon sources for the fermentative production of succinic acid, and in this aspect oat pomace could clearly be identified as suitable carbon source for *A. succinogenes*.

CRediT authorship contribution statement

Daniel Pleissner: Methodology, Investigation, Funding acquisition, Conceptualization. **Joachim Venus:** Methodology, Investigation. **Roland Schneider:** Methodology, Investigation. **Julia Dalichow:** Methodology, Investigation. **Judith Ettinger:** Methodology, Investigation. **Ralf Malchow:** Methodology, Investigation. **Corina Kleps:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Alemayehu GF, Forsido SF, Tola YB, Amare E. Nutritional and phytochemical composition and associated health benefits of oat (*Avena sativa*) grains and oat-based fermented food products. Sci World J 2023;2023:2730175.
[2] Buchanan D, Martindale W, Romeih E, Hebishy E. Recent advances in whey processing and valorisation: technological and environmental perspectives. Int J Dairy Technol 2023;76(2):291–312.
[3] Cui L, Jia Q, Zhao J, Hou D, Zhou S. A comprehensive review on oat milk: from oat nutrients and phytochemicals to its processing technologies, product features, and potential applications. Food Funct 2023;14(13):5858–69.
[4] Dessie W, Luo X, Duns GJ, Wang M, Qin Z. Towards the development of efficient, economic and environmentally friendly downstream processing for bio-based succinic acid. Environ Technol Innov 2023;32:103243.
[5] Kleps C, Schneider R, Venus J, Pleissner D. Investigation of the utilization of oat pomace and acid whey in technical scale succinic acid fermentation including downstream processing. Sustain Chem One World 2024;4:100028.
[6] Kuenz A, Hoffmann L, Goy K, Bromann S, Prüße U. High-level production of succinic acid from crude glycerol by a wild type organism. Catalysts 2020;10(5):470.
[7] Louasté B, Eloutassi N. Succinic acid production from whey and lactose by *Actinobacillus succinogenes* 130Z in batch fermentation. Biotechnol Rep 2020;27:e00481.
[8] Mao H, Xu M, Ji J, Zhou M, Li H, Wen Y, Wang J, Sun B. The utilization of oat for the production of wholegrain foods: processing technology and products. Food Front 2022;3(1):28–45.
[9] Mitrea L, Călinoiu L-F, Teleky B-E, Szabo K, Martău A-G, Ştefănescu B-E, Dulf F-V, Vodnar D-C. Waste cooking oil and crude glycerol as efficient renewable biomass for the production of platform organic chemicals through oleophilic yeast strain of *Yarrowia lipolytica*. Environ Technol Innov 2022;28:102943.
[10] Omwene PI, Yağcıoğlu M, Öcal-Sarihan ZB, Ertan F, Keris-Sen ÜD, Karagunduz A, Keskinler B. Batch fermentation of succinic acid from cheese whey by *Actinobacillus succinogenes* under variant medium composition. 3 Biotech 2021;11(8):389.
[11] Peinemann JC, Pleissner D. Continuous pretreatment, hydrolysis, and fermentation of organic residues for the production of biochemicals. Bioresour Technol 2020;295:122256.
[12] Pleissner D, Dietz D, van Duuren J, Wittmann C, Yang X, Lin CSK, Venus J. Biotechnological production of organic acids from renewable resources. Adv Biochem Eng/Biotechnol 2019;166:373–410.
[13] Pleissner D, Venus J. Agricultural residues as feedstocks for lactic acid fermentation. In: Green Technologies for the Environment. American Chemical Society; 2014. p. 247–63.
[14] Raj M, Devi T, Kumar V, Mishra P, Upadhyay SK, Yadav M, Sharma AK, Sehrawat N, Kumar S, Singh M. Succinic acid: applications and microbial production using organic wastes as low cost substrates. Phys Sci Rev 2023.
[15] Sterna V, Zute S, Brunava L. Oat grain composition and its nutrition benefice. Agric Agric Sci Procedia 2016;8:252–6.
[16] Stylianou E, Pateraki C, Ladakis D, Cruz-Fernández M, Latorre-Sánchez M, Coll C, Koutinas A. Evaluation of organic fractions of municipal solid waste as renewable feedstock for succinic acid production. Biotechnol Biofuels 2020;13(1):72.
[17] Tan JP, Md. Jahim J, Wu TY, Harun S, Kim BH, Mohammad AW. Insight into biomass as a renewable carbon source for the production of succinic acid and the factors affecting the metabolic flux toward higher succinate yield. Ind Eng Chem Res 2014;53(42):16123–34.
[18] Terboven C, Abendroth C, Laumer J, Herrmann C, Schneider R, Ramm P, Venus J, Plöchl M. Influence of the initial sugar concentration and supplementation with

yeast extract on succinic acid fermentation in a lactose-based medium. Fermentation 2021;7(4):221.
[19] Uysal U, Hamamcı H. Succinic acid production from cheese whey via fermentation by using alginate immobilized *Actinobacillus succinogenes*. Bioresour Technol Rep 2021;16:100829.
[20] Vallecilla-Yepez L, Wilkins MR. Continuous succinic acid production from corn fiber hydrolysate by immobilized *Actinobacillus succinogenes* in a hollow fiber membrane packed-bed biofilm reactor. Syst Microbiol Biomanuf 2023;3(4):765–75.
[21] Wan C, Li Y, Shahbazi A, Xiu S. Succinic acid production from cheese whey using *Actinobacillus succinogenes*, 130 Z. Totowa, NJ: Humana Press; 2008. p. 111–9.
[22] Xi Y-l, Chen K-q, Dai W-y, Ma J-f, Zhang M, Jiang M, Wei P, Ouyang P-K. Succinic acid production by *Actinobacillus succinogenes* NJ113 using corn steep liquor powder as nitrogen source. Bioresour Technol 2013;136:775–9.
[23] Yu J, Li Z, Ye Q, Yang Y, Chen S. Development of succinic acid production from corncob hydrolysate by *Actinobacillus succinogenes*. J Ind Microbiol Biotechnol 2010;37(10):1033–40.