

CONTINUOUS SUCCINIC ACID FERMENTATION BY
ACTINOBACILLUS SUCCINOGENES

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Abstract

Fermentations were performed in an external recycle bioreactor using CO₂ and D-glucose at feed concentrations of 20 and 40 g L⁻¹. Severe biofilm formation prevented kinetic analysis of suspended cell ('chemostat') fermentation, while perlite packing enhanced the volumetric productivity by increasing the amount of immobilised cells. The highest productivity of 6.35 g L⁻¹ h⁻¹ was achieved at a dilution rate of 0.56 h⁻¹. A constant succinic acid yield of 0.69±0.02 g/(g of glucose consumed) was obtained and found to be independent of the dilution rate, transient state and extent of biofilm buildup — approximately 53% of the carbon that formed phosphoenolpyruvate ended up as succinate. Byproduct analysis indicated that pyruvate oxidation proceeded solely via the formate-lyase pathway. Cell growth and corresponding biofilm formation were rapid at dilution rates higher than 0.35 h⁻¹ when the product concentrations were low (succinic acid < 10 g L⁻¹), while minimal growth was observed at succinic acid concentrations above this threshold.

Keywords: Continuous fermentation; Succinic acid; *Actinobacillus succinogenes*; Bioreactors; Biofilms; Glucose.

Introduction

In the past decade butanedioic acid or succinic acid (SA) has established itself as a forerunner in biorefinery platform chemicals. Four carbon dicarboxylic acids were already identified in the US Department of Energy's list (2004) of potential large-scale biomass-derived chemicals and in an updated report SA features as a standalone molecule [1,2]. It is therefore no surprise that commercial bioproduction of SA by companies such as BioAmber, Reverdia, Myriant Technologies and a joint venture between BASF and DSM-Purac is either already happening or in the construction phase. These four companies plan to produce SA in excess of 150,000 tons per annum by the end of 2015 [3,4].

Reactor design and operation of SA fermentation is likely to become more important with the prospect of bulk scale production on the horizon. Most of the current focus is on the development of the microorganism, with batch operation being the preferred mode of operation. Continuous production of SA is likely to outperform batch processing, especially when considering the projections of future processing quantities. In this regard the number of studies on continuous SA producing cultures is limited. The most documented SA producers are wild strains of *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Anaerobiospirillum succiniciproducens* and various recombinant strains of *Escherichia coli* [5,6]. These bacteria, in addition to recombinant *Corynebacterium glutamicum*, have been identified by McKinlay et al. [7] as the most promising SA producers. The wild strains are anaerobes that produce SA naturally as a major catabolic product via the phosphoenolpyruvate carboxykinase pathway [8]. Modified *E. coli* have been engineered to either copy the above-mentioned pathway with less byproduct formation [9] or produce SA aerobically via an interrupted TCA cycle [10]. The theoretical maximum yield is 1.71 mol SA per mol glucose (1.12 g g⁻¹) when redox requirements are considered and biomass formation is ignored. Only the wild SA-producing strains (*A. succinogenes*, *M. succiniciproducens* and *A. succiniciproducens*) have been studied under continuous conditions. With the exception of the work done by Urbance et al. [11] and Meynial-Salles et al. [12], all continuous SA work was done at the Korean Institute of Advanced Technology (KAIST) where *M. succiniciproducens* was first isolated [13–19]. Apart from normal suspended cell systems ('chemostat'), membrane systems with cell recycle as well as biofilm reactors were used in an attempt to enhance productivity [11–13,17]. Particular success was achieved by Meynial-Salles et al. [12] with *A. succiniciproducens* where a very high productivity of 14.8 g L⁻¹ h⁻¹ was obtained with a SA yield of 0.83 g g⁻¹. In addition to the cell recycle membrane reactor, a system was presented where an electro dialysis unit was utilised in conjunction with the reactor to remove organic acids in situ and thereby increase the final product titer up to 80 g L⁻¹ (at a productivity of 10.4 g L⁻¹ h⁻¹). Urbance et al. [11] also reported high productivities (up to 8.8 g L⁻¹ h⁻¹) by *A. succinogenes* where a special polypropylene composite support was used to enhance immobilisation. The results are, however, scattered with low SA yields at high productivities. Also, no details

on byproducts are provided. The chemostat data on *A. succiniciproducens* by Lee et al. [19] present a thorough set of data for two different glucose feed concentrations from which a consistently high yield over a broad range of dilution rates was obtained. A summary of continuous succinic acid fermentation studies is given in Table 1.

This work presents the third continuous study on *A. succinogenes* and includes a significant extension to the studies by Urbance et al. [11] and Kim et al. [13]. Emphasis is placed on the transient behaviour of the system, byproduct distribution and the potential advantages of utilising the immobilisation capabilities of *A. succinogenes*.

2. Materials and Methods

2.1 Microorganism

A. succinogenes 130Z (DSM 22257 or ATCC 55618) was acquired from the German Collection of Microorganisms and Cell Cultures (DSMZ). Vials containing treated beads in a cryopreservative solution were used to store culture samples at -75 °C. The inoculum was incubated at 38 °C and 100 rpm over a period of 20–24 h in 30 mL sealed bottles containing 15 mL tryptone soy broth (TSB).

2.2 Media composition

The medium was based on the formulations tested by Urbance et al. [20]. Chemicals were obtained from Merck KgaA (Darmstadt, Germany) unless indicated otherwise. The concentrations of the components in the medium were: 6 g L⁻¹ yeast extract, 10 g L⁻¹ corn steep liquor (Sigma-Aldrich, St. Louis, USA), 0.3 g L⁻¹ Na₂HPO₄, 1.4 g L⁻¹ NaH₂PO₄, 1.4 g L⁻¹ sodium acetate, 1 g L⁻¹ NaCl, 1.5 g L⁻¹ K₂HPO₄, 0.2 g L⁻¹ MgCl₂·6H₂O, 0.23 g L⁻¹ CaCl₂·2H₂O, 0.05 g L⁻¹ antifoam A (Sigma-Aldrich, St. Louis, USA) and 20 or 40 g L⁻¹ D-glucose. CO₂ (g) (African Oxygen, Johannesburg, South Africa) was used as the inorganic carbon source and was sparged through the reactor at approximately 0.05 vvm.

2.3 Fermentation

The bioreactor setup with an external recycle used for agitation is illustrated in Fig. 1.

Each reservoir was fitted with a 0.2 μm PTFE membrane filter (Midisart 2000 filters from Sartorius, Göttingen, Germany). The total open volume of fermentations was 156 mL and included the 'bioreactor' represented in Fig. 1 and the recycle line. The bioreactor consisted of an aluminium top and bottom section and a glass tube with a length of 115 mm and an inner diameter of 37.5 mm. The bottom section contained one entry/exit point for fermentation broth and also transferred heat from the hotplate to the broth. The top section contained an aluminium sheath that acted as a thermowell for the thermocouple and two additional entry/exit points for the broth. The connected, closed setups indicated by the dashed lines in Fig. 1 were sterilised in an autoclave at 121 °C for 40 min. The medium containing yeast extract, corn steep liquor and salts was autoclaved separately from the glucose solution and then mixed aseptically. The reactor was filled and operated at a stable temperature and pH before it was seeded with approximately 8–10 mL of the inoculum. 10 M non-sterile potassium hydroxide (KOH) was used to control the pH at 6.80 ± 0.05 . During startup the fermenter was operated at the lowest dilution rate that prevented the reactor from emptying through froth entrainment. This was done to approach batch conditions to allow for initial accumulation of biomass.

The same reactor setup was employed for the biofilm fermentations. Genulite™ Groperl (Infagro Natural Technologies, Johannesburg, South Africa) particles with equivalent diameters of 2–4 mm were added to the reactor to provide increased area for cell attachment. Groperl particles are expanded perlite particles consisting of amorphous volcanic glass. Due to geometric constraints, only 40% of the reactor's open volume could be filled with packing.

2.4 Analytical Methods

Glucose, ethanol and organic acid concentrations were determined by using high-performance liquid chromatography. An Agilent 1260 Infinity HPLC (Agilent Technologies, USA), equipped with an RI detector and a 300×7.8 mm Aminex HPX-87H ion-exchange column (Bio-Rad Laboratories, USA) was used. The mobile phase ($0.3 \text{ mL L}^{-1} \text{ H}_2\text{SO}_4$) was fed at a flowrate of 0.6 mL min^{-1} with a column temperature of

60 °C. Dry cell weight (DCW) was determined from 4.5 mL samples centrifuged at 12 100 g for 5 min. Cell pellets were washed twice with distilled water and dried at 90 °C for at least 24 h.

2.5 Sampling and data analysis

Mass balance checks were performed for all samples obtained after five volume turnovers at a constant dilution rate. Dry cell masses varied between 0.7 and 1.4 g L⁻¹, except when biofilm chunks were present in the sample. This occurred frequently, but the specific data were ignored in the mass balance calculations. The glucose feed concentration was estimated with a mass balance by considering all the outlet measurements. It was found that the estimated glucose feed concentration was always 95-102% of the actual make-up concentration with an average value of 97%. This lower value can be attributed to the dilution effect of the KOH dosing since the average dosing flowrate accounted for 2–3% of the total dilution rate (based on outlet flow conditions). Cellular mass uptake of essential amino acids and vitamins from the yeast extract and corn steep liquor was assumed to be negligible in terms of the total mass balance.

The KOH (aq) dosing was related to the productivity of the acid products by assuming complete dissociation of succinic, formic and acetic acid. The molar rate at which hydroxide should be fed can be determined by Eq. (1):

$$F_{KOH} = \left(2 \frac{P_{SA}}{M_{SA}} + \frac{P_{AA}}{M_{AA}} + \frac{P_{FA}}{M_{FA}} \right) V \quad (1)$$

The time-dependent KOH (aq) dosing flowrate was generated continuously by calculating time-averaged dosing flowrates based on 1 h intervals and the dosing pump calibrations. This allowed online monitoring of the total acid productivity and Eq. (1) was used to compare the prediction with the results from the HPLC.

3. Results and Discussion

3.1 Biofilm Formation and Productivity

The initial objective was to quantify the kinetics of *A. succinogenes* through suspended cell or 'chemostat' fermentation. This is necessary to determine the specific growth rate and the substrate and product inhibition characteristics of *A. succinogenes* under continuous culture conditions employing the specified medium. For this reason non-packed vessel fermentations were performed. However, all non-packed vessel fermentations clearly indicated that chemostat conditions were impossible to achieve due to severe attachment of cells on the glass walls and internals. Urbance et al. [11] proved that *A. succinogenes* was naturally capable of biofilm formation, although the extent to which the cells were capable of adhering to glass and aluminium surfaces was unexpected. The recycle rate was increased from 100 to 300 mL min⁻¹ in order to increase the shear conditions inside the reactor to remove the biofilms, but no success was achieved. The effect of the gradual increasing amount of attached cells in the bioreactor is illustrated in Fig. 2. where the SA productivity increases from 6 g L⁻¹ to 13 g L⁻¹ over 27 volume turnovers, while the maximum productivity of 1.8 g L⁻¹ h⁻¹ is achieved at the end of the fermentation. The assumption of five volume turnovers for a chemostat to achieve steady state [21] clearly does not apply since the volumetric production rate of SA is still increasing after 27 volume turnovers. Since biofilm formation could not be stopped, Groperl support was added to the fermenter to increase the area of immobilisation. The results from a fermentation performed at a dilution rate of $D=0.56\text{ h}^{-1}$ can be seen in Fig. 3. From the productivity values obtained in this fermentation (greater than 6 g L⁻¹ h⁻¹) it is evident that the amount of immobilised cells in a packed reactor must be more than in a non-packed reactor. For the fermentation illustrated in Fig. 3 steady state was achieved at approximately 30 volume turnovers with a final SA concentration of 12 g L⁻¹.

For all fermentations performed contamination occurred during attempted feed reservoir changes. The formation of lactic acid was used as indicator for infection and all fermentations were terminated after contamination occurred. No data from infected fermentations were used. Total cell counts (biofilm and suspended cells) of the pure

culture could not be performed due to the infections and accordingly a kinetic analysis was not possible. Performance was tested at various dilution rates. Steady state could not be confirmed for all the measurements due to the single feed reservoir constraint. These pseudo-steady state measurements provided insight into the effect of the product concentrations on biofilm formation. Rapid biofilm formation was visually observed at high dilution rates (larger than 0.35 h^{-1}) indicating high growth rates at low product concentrations. Correspondingly, very slow biofilm formation was observed at lower dilution rates.

Various pseudo-steady state productivity measurements are given in Fig. 4 as a function of dilution rate. The variations in productivity at a certain dilution rate are linked to the amount of attached biomass present in the reactor. At a glucose feed concentration of 20 g L^{-1} with the dilution rates below 0.21 h^{-1} in the non-packed reactor, glucose conversion was close to completion. For the higher dilution rates the productivity was found to be a function of the accumulative time that the reactor was operated above a dilution rate of 0.35 h^{-1} . In this rapid growth phase ($D > 0.35 \text{ h}^{-1}$) biofilm maturity was achieved after approximately 48 h of operation and the markers representing these conditions give the maximum productivity achieved. It is evident that the packed bed reactor outperforms the non-packed vessel due to the extent of cell immobilisation. A maximum productivity of $6.35 \text{ g L}^{-1} \text{ h}^{-1}$ was obtained at a dilution rate of 0.56 h^{-1} . This highlights the potential of utilising the biofilm-formation capability of *A. succinogenes*.

3.2 SA Yield

The SA yield was relatively constant for all fermentations with values ranging from 0.67 to 0.71 g g^{-1} . The samples analysed were always in excess of four turnovers after a change in dilution rate. Only SA, acetic acid and formic acid were formed in significant amounts. The distribution of product formation is presented in Fig. 5 and Fig. 6. Ethanol formation was always less than 0.08 g L^{-1} , while no lactic acid formation was detected. It is evident from Fig. 5 that the ratio of SA to acetic acid in the effluent is fairly constant, irrespective of the amount of biofilm formed and the transient behaviour of the system (steady state was not achieved for most samples). The least squares fit through the

origin presented in Fig. 6 indicates a concentration ratio of SA to acetic acid of 2.5 to 1 on a mass basis. Given the molar masses of these components, the carbon fixation associated with SA and the CO₂ formation that accompanies the formation of acetic acid it can be shown that 53% of the carbon that reaches phosphoenolpyruvate (PEP) is used to produce succinate in the reverse TCA cycle, while pyruvate is produced from the rest. For a comprehensive illustration of the metabolic pathways of *A. succinogenes* refer to McKinlay et al. [22].

Fig. 6 gives the relation between the amounts of acetic and formic acid produced. The trend line represents equimolar amounts of the two main byproducts, and the data closely follow the trend. This equimolar distribution indicates that pyruvate oxidation proceeded via the formate-lyase pathway (pyruvate formate-lyase enzyme) and that no NADH is produced in the acetyl-CoA formation step. This is unfavourable in terms of the SA yield, where the reduction requirements of the reverse TCA cycle (succinate branch) dictate the split at the PEP node. Although both the pyruvate and formate dehydrogenase enzymes are reported to be part of the catabolism of *A. succinogenes* [22–25], they appear to be inactive in the experimental results presented. The benefit of their activity is illustrated by the high SA yields obtained by *A. succiniciproducens* (greater than 0.8 g g⁻¹) where no formic acid is formed [12,18].

3.3 Cell Growth Termination

Since measurements of the specific growth rate and reactor biomass concentration were not available, a cell-based analysis of the reactor was not feasible. Nevertheless, certain trends were observed that were in close agreement with the detailed batch kinetic study of Corona-González et al. [26]: the data from their fermentations suggested that a critical SA titer (approximately 11–13 g L⁻¹) was reached in each fermentation where cell growth terminated completely. The inhibition is most likely caused by the combination of all acids present in the broth, but for this discussion the SA titer will be used as indicator (given the constant product distribution). In this study it was also observed that cell growth (and the corresponding rate of cell attachment) was rapid at high dilution rates when the SA titer was below approximately

10 g L⁻¹, while higher SA titers resulted in no observable increases in cell immobilisation. It is also plausible that a significantly high concentration of the cation of the neutralising base is responsible for the termination, according to studies by Liu et al. [27]. The data from Corona-González et al. [26] also indicated that the growth termination point had no significant influence on the production of SA if excess glucose was available – the rate of SA production remained more or less constant before and after the cell growth termination point. This suggests that the energy requirements for cell maintenance are significant at high SA titers and that the non-growing cells are metabolically active.

The maintenance-driven production of SA can be used to interpret the continuous results obtained in this study. The complete set of results for one of the non-packed vessel fermentations are given in Fig. 7. In this fermentation the time-averaged KOH dosing profile is shown and compared with the dosing requirements based on the total acid production suggested by results obtained via HPLC analysis. From Fig. 7 it is evident that the online monitoring of productivity was possible. Similar to all the other fermentations, a steady increase in total acid productivity with time is observed as increasing amounts of cells gradually attach to the inside of the reactor. An interesting observation can be made when the dilution rate is altered from 0.27 h⁻¹ to 0.16 h⁻¹: a two-fold increase in SA concentration can be seen, while the productivity remains almost constant. If it is assumed that there is no growth during the period when the dilution rate is at 0.16 h⁻¹ (SA titer at 13 g L⁻¹), the effect of the acid concentrations on the maintenance production of SA can be taken as minor, where the maintenance production rate is mainly a function of the amount of cells in the reactor. The gradual washout of non-growing cells will result in a productivity decrease, although this was not observed in five volume turnovers at a dilution rate of 0.16 h⁻¹. It is also evident from Fig. 7 that cell growth commences during the last two phases of the fermentation when the dilution rate is increased to 0.38 and 0.68 h⁻¹ – the increase in dilution rate is accompanied by a decrease in SA titer to below 10 g L⁻¹. The KOH dosing plateau obtained at the end of the 0.38 h⁻¹ section is at an SA titer of 8.5 g L⁻¹, which is slightly lower than the suggested 10 g L⁻¹ for growth termination. Stabilisation of the dosing

profile at a dilution rate of 0.68 h^{-1} (not reached during the fermentation) will be as a result of either an SA titer close to 10 g L^{-1} or a matured biofilm in which the biomass content in the reactor has reached a maximum.

3.4 Literature comparison

In Fig. 8 the results from this study are compared with the continuous results of Urbance et al. [11] – all data shown were obtained from fermentations where the glucose feed concentration was 20 g L^{-1} . Fig. 8 allows comparison between productivity, glucose consumption rate, yield and conversion at different dilution rates. It can be noted that apart from the higher productivities achieved in this study, the SA yields are more consistent and generally higher (shorter bars represent higher yields). The ultimate aim, in terms of Fig. 8, is to push the markers up while decreasing the length of the bars. If possible, this should be done at higher glucose concentrations to allow higher SA titers.

4. Conclusion

This study provided valuable insights into the continuous production of SA with *A. succinogenes*. The immobilisation characteristics of the culture under continuous conditions made chemostat operation impossible, but resulted in high volumetric productivities when the attachment area was increased with packing. Productivities in excess of $6 \text{ g L}^{-1} \text{ h}^{-1}$ were obtained by filling only 40% of the fermenter volume with packing, suggesting that further improvements are possible.

Proper yield quantification was performed with mass balances calculated for each reported sample. The overall SA yield varied between 0.67 and 0.71 g g^{-1} , while a constant product distribution was observed. Analysis of acetic and formic acid production ratios indicated that pyruvate oxidation occurred solely via the formate-lyase route. It was shown that 53% of the PEP carbon ended up as SA. Interestingly, these narrow yield and product distribution results were unaffected by dilution rate, fermenter transient state or the amount of cells immobilised in the growing biofilm.

Contamination prevented a proper kinetic analysis, although growth inhibition characteristics could be observed. It is evident that a certain product concentration exists where growth terminates, while SA production continues.

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Nomenclature

| | |
|-----|---|
| C | Concentration/titer (g L^{-1}) |
| D | Dilution rate (h^{-1}) |
| F | Molar flowrate (mol h^{-1}) |
| M | Molar mass (g mol^{-1}) |
| P | Volumetric productivity ($\text{g L}^{-1} \text{h}^{-1}$) |
| V | Volume of reactor (L) |
| Y | Yield (g g^{-1}) |

Subscripts

| | |
|----|---------------|
| S | Substrate |
| SA | Succinic acid |

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Figure captions

Fig. 1. Schematic representation of continuous bioreactor setup. Fermentation broth exited the reactor by overflow.

Fig. 2. Eight day continuous operation at $D=0.14 \text{ h}^{-1}$ in a non-packed reactor with a glucose feed concentration of 40 g L^{-1} . Succinic acid concentration (o), glucose concentration (+), volumetric productivity of succinic acid (A), succinic acid yield (x).

Fig. 3. 78 h Continuous operation at $D=0.56 \text{ h}^{-1}$ with perlite packing in the bioreactor with a glucose feed concentration of 20 g L^{-1} . Succinic acid concentration (o), glucose concentration (+), volumetric productivity of succinic acid (A), succinic acid yield (x).

Fig. 4. Volumetric productivity of succinic acid at various pseudo-steady states. The glucose feed concentration was 20 g L^{-1} . Productivity when perlite packing was included in the reactor (D), non-packed vessel productivity at low dilution rates (A), non-packed vessel productivity at dilution rates higher than 0.35 h^{-1} for periods shorter than 48 h (o), non-packed vessel productivity at dilution rates higher than 0.35 h^{-1} when operation exceeded 48 h (\bullet), maximum productivity when the yield is 0.7 (-).

Fig. 5. Mass ratio of succinic acid to acetic acid for all samples (o), 2.5 g succinic acid produced per gram of acetic acid produced (-).

Fig. 6. Mass ratio of acetic acid to formic acid for all samples (o), equimolar amounts of acetic acid and formic acid (-).

Fig. 7. An non-packed vessel fermentation with a glucose feed concentration of 20 g L^{-1} . Glucose concentration (+), succinic acid concentration (o), acetic acid concentration (D), formic acid concentration (0), dry cell concentration (x), actual molar flowrate of KOH to the reactor (-), predicted molar flowrate of KOH needed to control the pH in the reactor (A).

Fig. 8. Comparison of volumetric reaction rates obtained in this study with those obtained by Urbance et al. [10]. Volumetric productivity of succinic acid in this study (D), suspended cell fermentations agitated at 125 RPM by Urbance et al. [10] (o),

biofilm grown on polymer composite support and agitated at 125 RPM (D), glucose consumption rates corresponding to productivities (+), maximum productivity when the yield is 0.7 (–), glucose consumption rate at full glucose conversion (--).

Table 1

Summary of continuous succinic acid fermentation studies

| Microorganism | Substrate (g L ⁻¹) | D (h ⁻¹) | Reactor type | Max P _{SA} (g L ⁻¹ h ⁻¹) | Y _{SA/S} (g g ⁻¹) | Max C _{SA} (g L ⁻¹) | Reference |
|---|-----------------------------------|-------------------------|-------------------------|---|---|---|-----------|
| <i>A. succinogenes</i> 130Z | Glucose: 20 | 0.2-1.2 | Suspended cell, biofilm | 8.8 | 0.27-0.73 | 10.4 | [11] |
| <i>A. succinogenes</i> 130Z | Glucose: 60 | 0.2-0.5 | Cell recycle | 6.63 | 0.50-0.59 | 18.6 | [13] |
| <i>A. succiniciproducens</i> ATCC No. 29305 | Lactose: 45 | 0.085-0.15 | Suspended cell | 3.0 | 0.62-0.72 | 24.0 | [28] |
| <i>A. succiniciproducens</i> ATCC No. 29305 | Lactose: 20 | 0.03-0.14 | Suspended cell | 1.4 | 0.81-0.94 | 14.0 | [16] |
| <i>A. succiniciproducens</i> ATCC No. 53488 | Glucose: 20 | 0.19-0.93 | Cell Recycle | 14.8 | 0.74-0.83 | 16.5 | [17] |
| <i>A. succiniciproducens</i> ATCC No. 29305 | Glucose: 19, 38 | 0.032-0.63 | Suspended cell | 6.5 | 0.73-0.82 | 29.6 | [18] |
| <i>A. succiniciproducens</i> ATCC No. 29305 | Glycerol: 10.7, 11, 11.3 | 0.022-0.25 | Suspended cell | 2.2 | 1.23-1.50 | 16.1 | [19] |
| <i>B. succiniciproducens</i> DD1 | Glycerol: 5.1 | 0.004-0.018 | Suspended cell | 0.0094 | 0.71-1.02 | 5.2 | [29] |
| <i>M. succiniciproducens</i> MBEL55E | Lactose: 21 | 0.1-0.7 | Suspended cell | 3.9 | 0.63-0.69 | 10.3 | [15] |
| <i>M. succiniciproducens</i> MBEL55E | Glucose & xylose: 18, 7 | 0.1-0.7 | Suspended cell | 3.2 | 0.34-0.61 | 14.1 | [14] |
| <i>M. succiniciproducens</i> LPK7 | Glucose: 9, 18 | 0.1-0.3 | Suspended cell | 1.77 | 0.10-0.71 | 12.9 | [30] |
| <i>M. succiniciproducens</i> MBEL55E | Glucose: 20 | 0.1-0.3 | Cell Recycle | 2.85 | 0.48-0.64 | 12.8 | [13] |

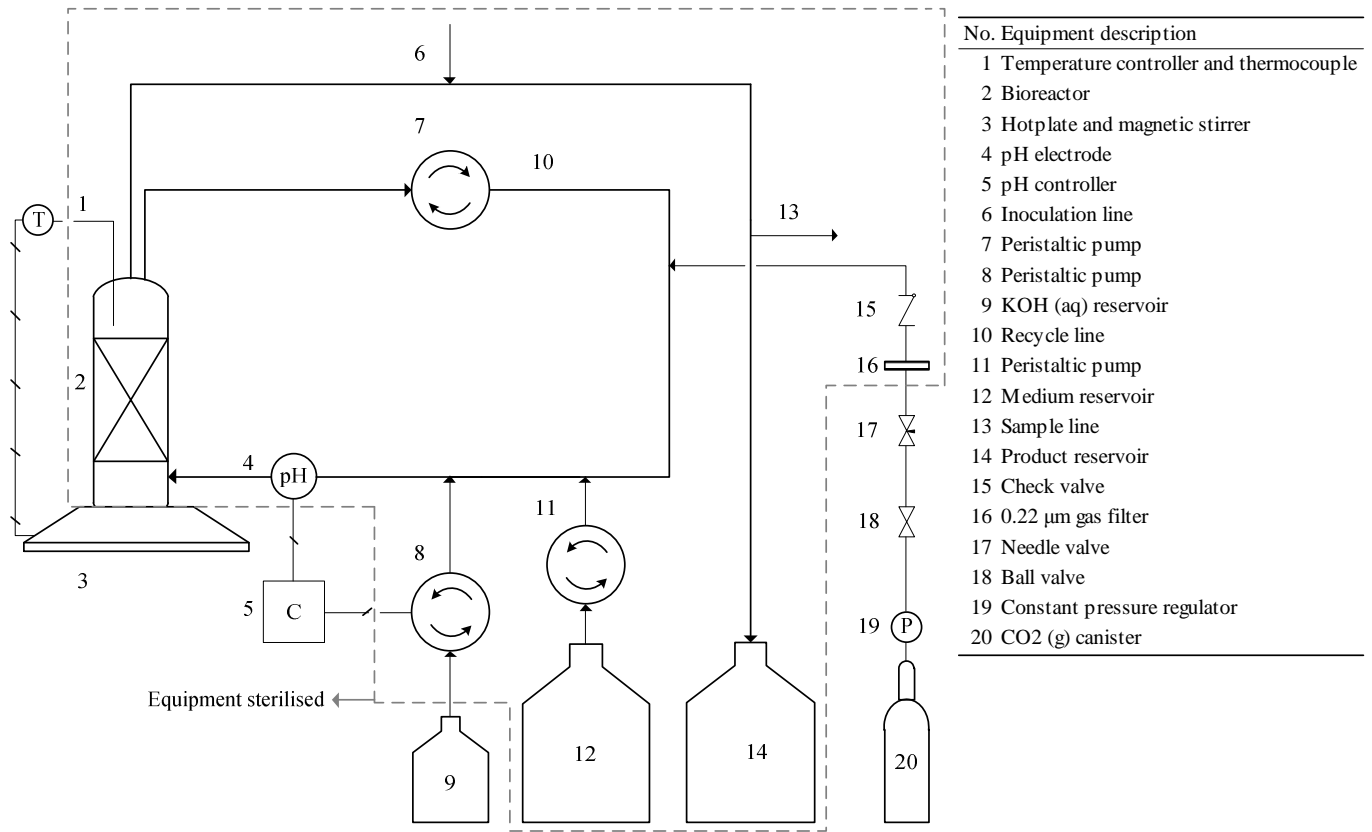


Fig. 1. Schematic representation of continuous bioreactor setup. Fermentation broth exited the reactor by overflow.

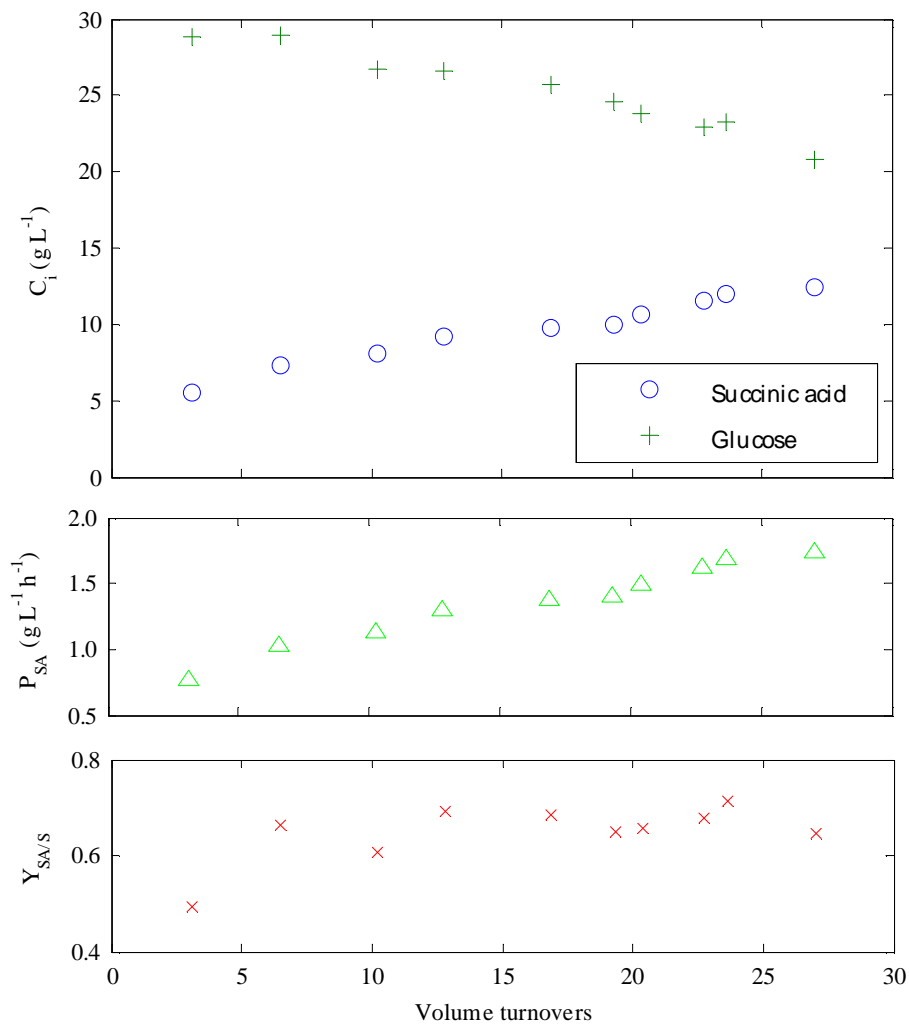


Fig. 2. Eight day continuous operation at $D=0.14 \text{ h}^{-1}$ in a non-packed reactor with a glucose feed concentration of 40 g L^{-1} . Succinic acid concentration (o), glucose concentration (+), volumetric productivity of succinic acid (Δ), succinic acid yield (x).

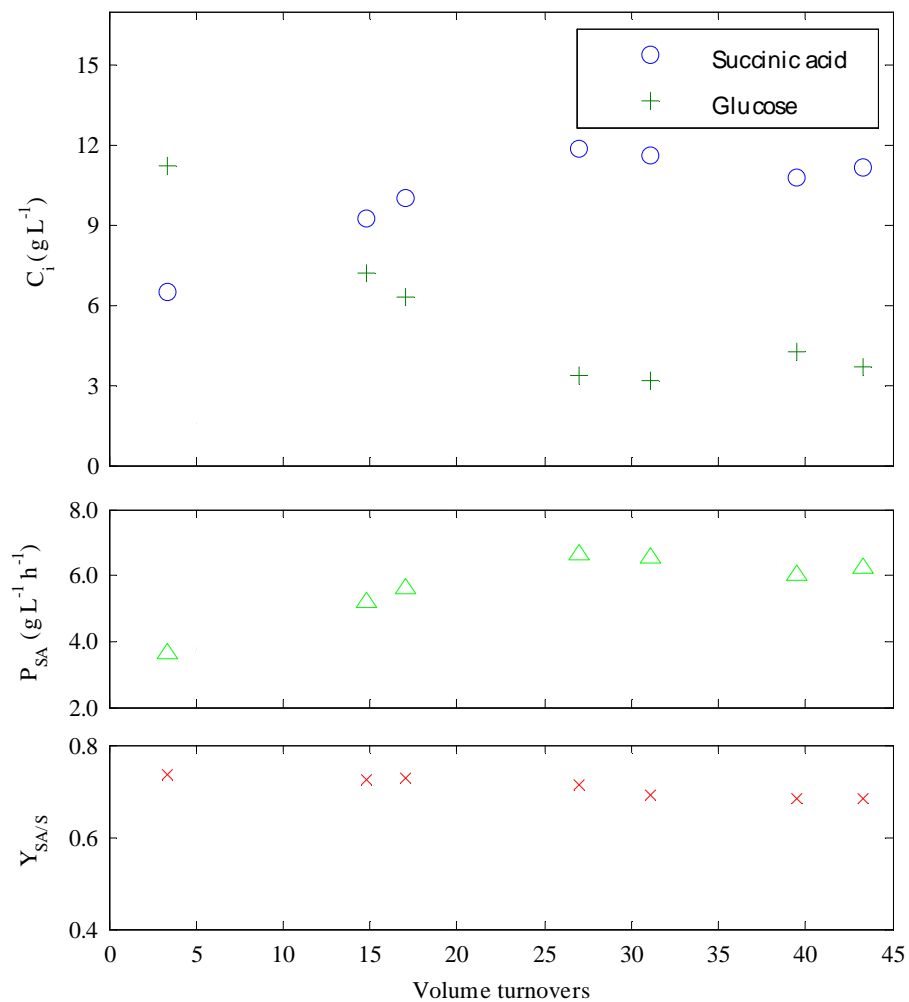


Fig. 3. 78 h Continuous operation at $D=0.56 \text{ h}^{-1}$ with perlite packing in the bioreactor with a glucose feed concentration of 20 g L^{-1} . Succinic acid concentration (o), glucose concentration (+), volumetric productivity of succinic acid (Δ), succinic acid yield (x).

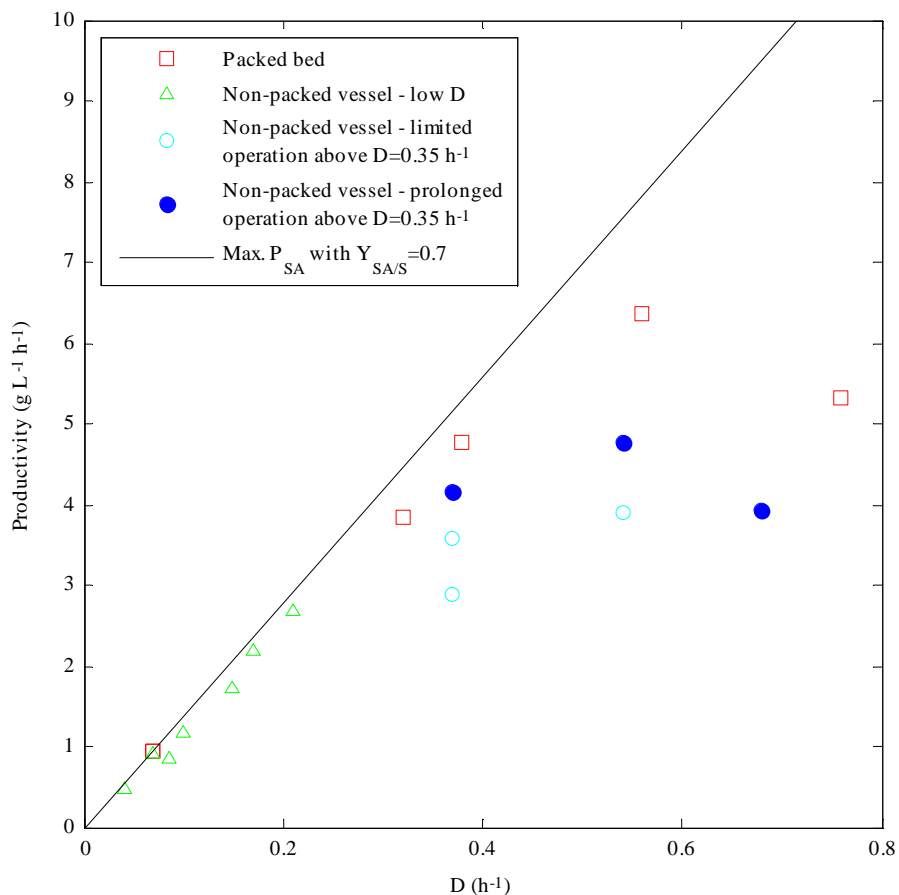


Fig. 4. Volumetric productivity of succinic acid at various pseudo-steady states. The glucose feed concentration was 20 g L⁻¹. Productivity when perlite packing was included in the reactor (□), non-packed vessel productivity at low dilution rates (Δ), non-packed vessel productivity at dilution rates higher than 0.35 h⁻¹ for periods shorter than 48 h (○), non-packed vessel productivity at dilution rates higher than 0.35 h⁻¹ when operation exceeded 48 h (●), maximum productivity when the yield is 0.7 (—).

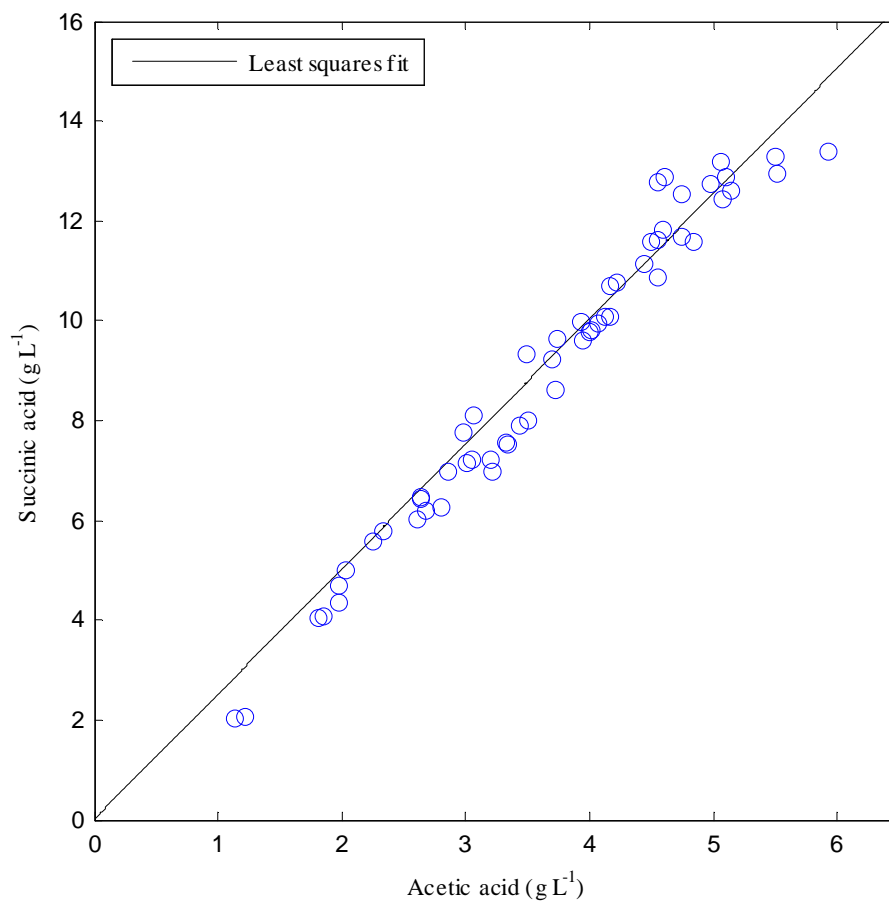


Fig. 5. Mass ratio of succinic acid to acetic acid for all samples (o), 2.5 g succinic acid produced per gram of acetic acid produced (—).

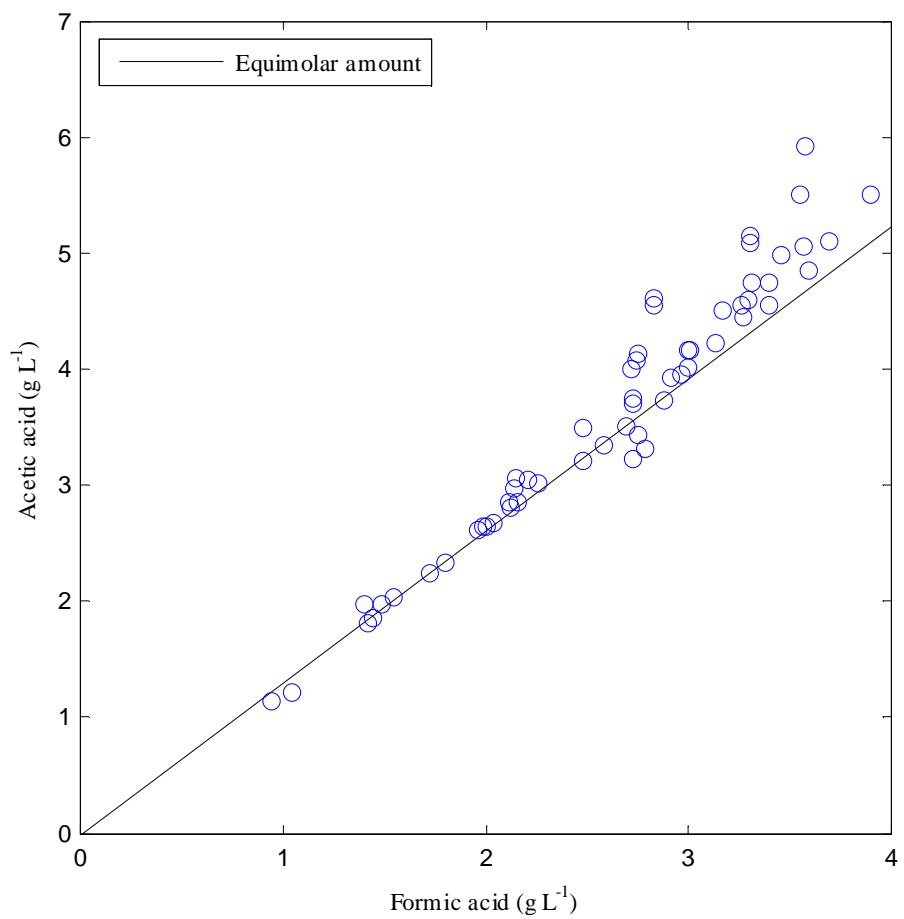


Fig. 6. Mass ratio of acetic acid to formic acid for all samples (o), equimolar amounts of acetic acid and formic acid (-).

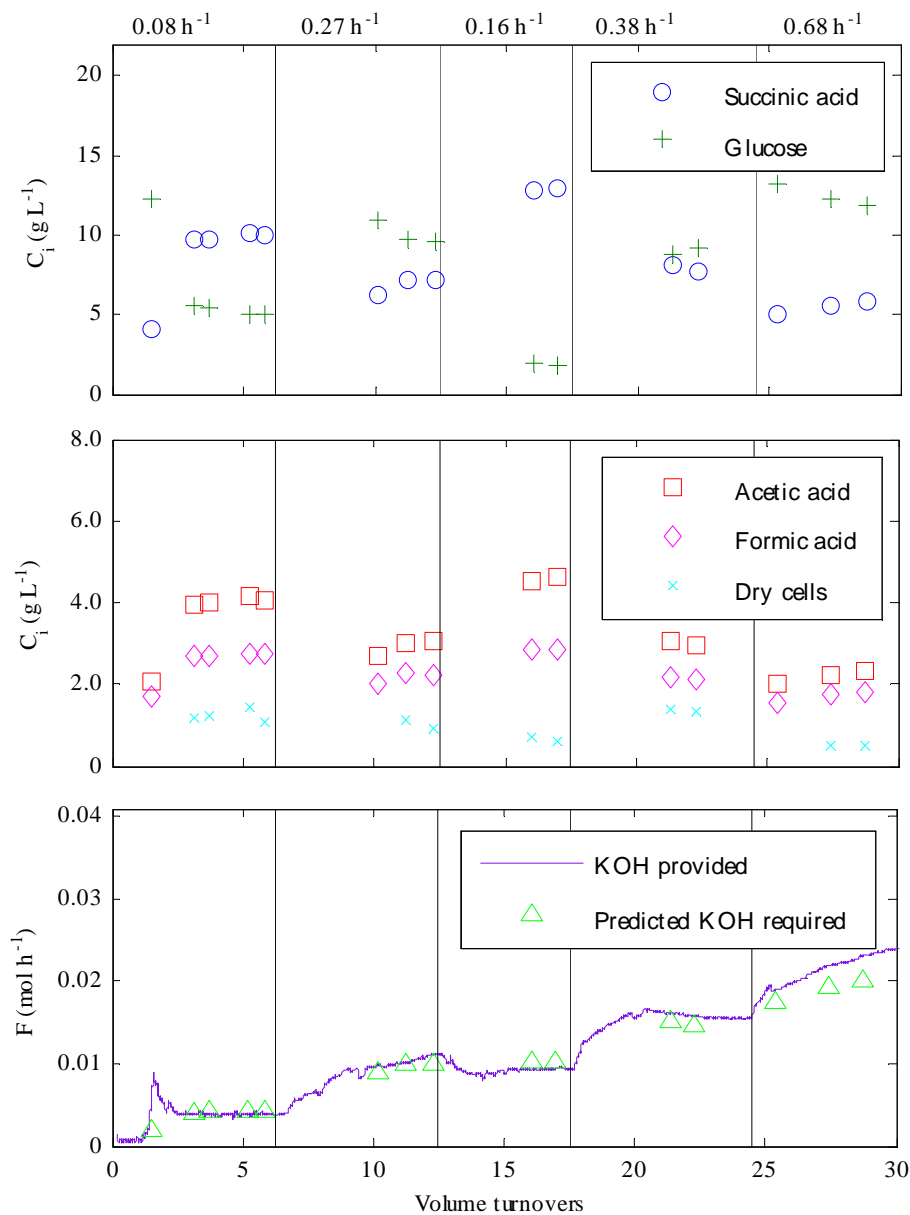


Fig. 7. An non-packed vessel fermentation with a glucose feed concentration of 20 g L⁻¹. Glucose concentration (+), succinic acid concentration (o), acetic acid concentration (□), formic acid concentration (◇), dry cell concentration (x), actual molar flowrate of KOH to the reactor (—), predicted molar flowrate of KOH needed to control the pH in the reactor (Δ).

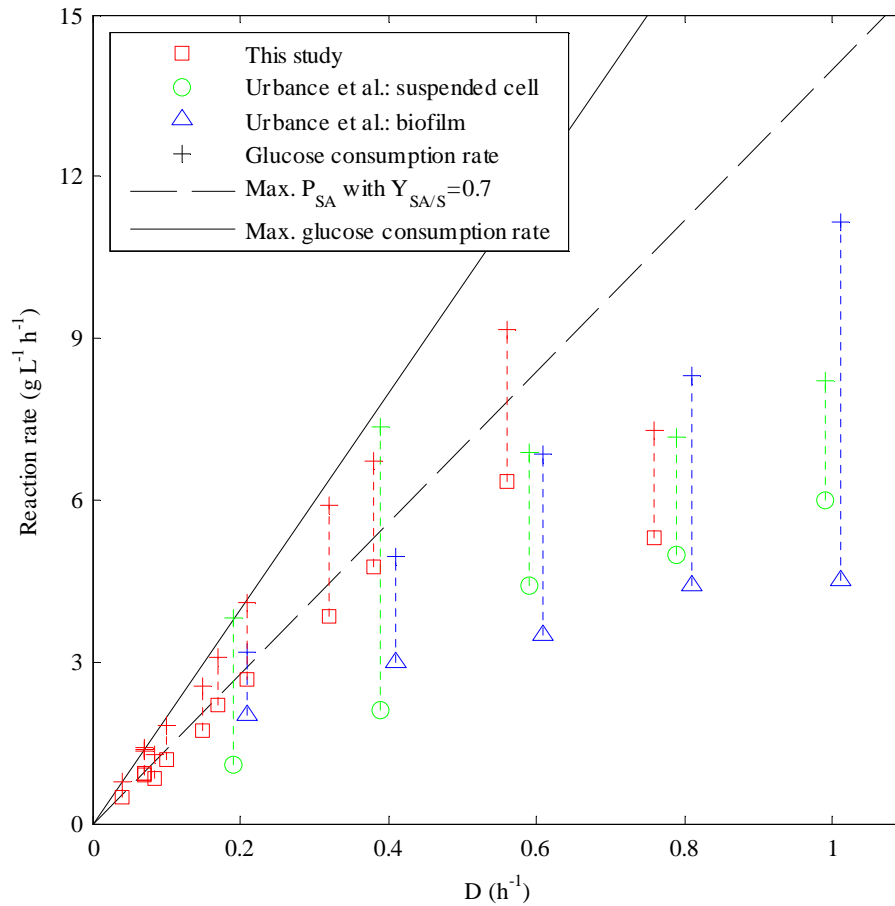


Fig. 8. Comparison of volumetric reaction rates obtained in this study with those obtained by Urbance et al. [10]. Volumetric productivity of succinic acid in this study (□), suspended cell fermentations agitated at 125 rpm by Urbance et al. [10] (○), biofilm grown on polymer composite support and agitated at 125 rpm (△), glucose consumption rates corresponding to productivities (+), maximum productivity when the yield is 0.7 (–), glucose consumption rate at full glucose conversion (--).