Effect of the process conditions on the anaerobic fermentation of glucose for the production of chemicals

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Abstract

In the context of biorefinery for the production of chemicals, this study optimised the processes to enhance the anaerobic production of ethanol and short chain organic acids from glucose. The optimised variables included (with a total of 49 runs) residence time (2-100 h), temperature (25-35 ^{O}C), type of inoculum (soil or anaerobic digester), presence or absence of pre-acclimation of the inoculum, glucose concentration (5-50 g/L), continuous or batch mode and composition of the mineral media (mineral solution with or without trace elements, with deionised or tap water). In continuous experiments the residence time was the most important parameter that affected glucose conversion (over 80 % glucose conversion for residence time longer than 30 h) and product yields (ethanol was the main product in the range of residence times 20-50 h, with yields in the range 0.30-0.40 g/g glucose removed). Temperature, type of inoculum and pre-acclimation had little effect on glucose conversion and products yield. The addition of trace elements had an important beneficial effect on the removal of glucose when it was fed at the highest concentration (50 g/L) in both continuous and batch experiments. In batch runs acetate was generally the main fermentation product rather than ethanol and ethanol conversion into acetate was favoured by nitrogen sparging, probably due to the reduced hydrogen partial pressure.

Keywords: glucose, anaerobic fermentation, biorefinery, organic acids, ethanol.

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Introduction

Anaerobic digestion of organic waste and biomass is carried out at commercial scale for the production of methane, a useful process for the production of renewable energy. However, in recent years the use of anaerobic digestion (or anaerobic fermentation) to produce chemicals, such as short-chain organic acids (e.g. acetic, propionic, butyric), alcohols (mainly ethanol) and hydrogen is of increasing interest [1, 2]. Organic acids, ethanol and hydrogen are important chemicals with a wide range of uses in the chemical industry or as fuels. However, currently they are mainly produced from fossil fuels or from food crops (e.g. bioethanol production from corn or sugarcane), which limits their sustainability [3]. On the other hand, short-chain organic acids, ethanol and hydrogen are intermediates in anaerobic digestion and could be obtained from organic waste and biomass if the process conditions are controlled to prevent further conversion of these intermediates to methane. Production of these chemicals from the anaerobic fermentation of organic waste or biomass, which are renewable resources, would enhance the sustainability of their production.

The conversion of organic waste and biomass into short chain organic acids, ethanol and hydrogen depends on managing many parameters that include the nature of the waste/biomass and the operating conditions (residence time, pH, temperature, etc). Several studies have investigated the effect of operating conditions on acidogenic digestion of organic waste (e.g. [4-6]). However, these studies were carried out with complex organic waste and, although they are very valuable for practical applications, don’t allow to understand the fate of the individual components of the waste in the

process. More experimental investigation is therefore needed to understand the acidogenic fermentation of the main chemical constituents of the waste.

Glucose is a main building block of organic waste and biomass and is present in carbohydrates as free sugar or as starch and cellulose. This is pertinent to study because carbohydrates make up over 50 % of the dry weight of many types of organic waste, e.g. the organic fraction of municipal solid waste (OFMSW), manure, energy crops and agricultural residues [7]. Several literature studies have been reported on the acidogenic fermentation of glucose for the production of chemicals using open mixed cultures [8-10]. However, all these studies were carried out with low glucose concentrations (lower than 10 g/L) and with a limited range of residence times. The main novelty of this study is that we have extended the range of operating conditions investigated for the anaerobic fermentation of glucose, generating data on how to choose and optimise the process conditions to obtain the desired product(s). We worked at higher concentrations (up to 50 g/L) than other reported studies and in a wider range of residence times (in the range 1-100 h). Furthermore, we investigated the effect of the composition of the mineral medium and of the type of inoculum used and of its acclimation to the substrate. We investigated the effect of these fermentation parameters on glucose removal and on the yield and composition of the fermentation products (organic acids and alcohols) with the aim of optimising the fermentation conditions for the desired products in a biorefinery context.

Materials and methods

Chemicals and inoculum

The composition of the standard mineral medium (MS) used was the following (g/L): $C_6H_{12}O_6$ (glucose) 5-50; K_2HPO_4 (potassium phosphate dibasic) 69.6; NaH_2PO_4 (sodium phosphate monobasic) 48; $CaCl_2 \cdot 6H_2O$ (calcium chloride hexahydrate) 0.09; $MgCl_2 \cdot 6H_2O$ (magnesium chloride hexahydrate) 0.125; NH_4Cl (ammonium chloride) 2. In some runs, other mineral media were also added to the standard medium MS. M9 minimal salt medium (M9, g/L): Na_2HPO_4 (sodium phosphate dibasic) 33.9; KH_2PO_4 (potassium phosphate monobasic) 15; NH_4Cl (ammonium chloride) 2; NaCl (sodium chloride) 2.50. Trace element solution (TE, g/L except for the Metals solution): $C_6H_9NO_6$ (nitriloacetic acid) 20; $MgSO_4 \cdot 7H_2O$ (magnesium sulphate heptahydrate) 28.9; $CaCl_2 \cdot 2H_2O$ (calcium chloride dihydrate) 6.67; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (ammonium heptamolybdate tetrahydrate) 0.03; $FeSO_4 \cdot 7H_2O$ (iron(II) sulphate heptahydrate) 0.20; Metals solution 100 ml/L. Metals solution (g/L): $C_{10}H_{14}N_2Na_2O_8$ (EDTA di-sodium salt) 2.50; $ZnSO_4 \cdot 7H_2O$ (zinc-sulphate heptahydrate) 10.95; $FeSO_4 \cdot 7H_2O$ (iron(II) sulphate heptahydrate) 5; $MnSO_4 \cdot H_2O$ (manganese(II) sulphate monohydrate) 1.54; $CuSO_4 \cdot 5H_2O$ (copper(II) sulphate pentahydrate) 0.39; $Co(NO_3)_2 \cdot 6H_2O$ (cobalt(II) nitrate hexahydrate) 0.25; $NaB_4O_7 \cdot 10H_2O$ (sodium tetraborate decahydrate) 0.18. When used, the M9 and TE media were added to the MS medium at 10 mL/L. The media were prepared in deionised (reverse osmosis) water except for a few runs in which tap water was used. All chemicals were of analytical grade and purchased from either Fisher Scientific or Sigma Aldrich.

Two inocula were used in this study. The first inoculum was soil (Craibstone soil) obtained from an agricultural site, Scotland Rural College's Estate, Craibstone Estate, Aberdeen,

Scotland, United Kingdom. The second inoculum was a sludge solution obtained from a mixed-substrate anaerobic digestion (AD) plant, Gask Farm Biogas Plant, near Turriff in Aberdeenshire, Scotland, United Kingdom. Sludge and soil were stored and maintained in a refrigerator at 4 ^{O}C prior to use and were collected every 6 months.

Reactors set-up and operation

The runs were carried out in glass reactors stirred with a magnetic bar at 200 rpm. Water was recirculated through a water jacket to maintain constant temperatures of 25^{0}C ± 1^{0}C or 35^{0}C ± 1^{0}C. pH was not controlled but, due to the use of the phosphate buffer in the mineral solution, it remained in the range 6.3 – 6.8 in all the runs. In continuous-flow runs the feed tank containing was connected through a tube and fed through Lambda Preciflow or Velp SP pumps. The effluent was removed using an overflow tube and collected before disposal.

To start up the continuous-flow reactors, in each reactor 2 g (1.19 g VSS/L of inoculum concentration) of Craibstone topsoil or 10 ml (1.35 g VSS/L of inoculum concentration) of digester sludge were inoculated and the mineral solution was added to reach a volume of 200 mL. Nitrogen was initially used to sparge each reactor for 30 minutes and then the feed pump was started at the desired flow rate. The residence time was controlled by controlling the feed flow rate. In all the runs, except in those with pre-accclimation, the reactors were started without any pre-acclimation of the inoculum with the substrate. In the runs with pre-acclimation the inoculum was maintained in contact with the substrate for one week before the feed pump was started. Each continuous run was operated until a steady state was achieved, which typically required between 15 and 100 d. The steady state was considered to be achieved when the concentration of the measured species

(glucose, biomass and products) did not vary by more than 20 % for 5 consecutive days. At the end of each run, each reactor was cleaned and a new run was started with a fresh inoculum, fresh mineral solution and nitrogen sparging as described above. Each reactor was monitored for volatile suspended solids (VSS, assumed to represent the biomass concentration), glucose, pH, ethanol, acetic acid and other volatile fatty acids (VFAs). Based on the measured values, steady state values and standard deviation for each variable and each run were calculated as the average and standard deviation of the values measured after steady state was reached. Batch reactors were inoculated, operated and monitored in the same way as the continuous reactors, with the difference that there was no feed pumped into the reactors and no effluent withdrawal.

A total of 49 runs was carried out (Table 1) by varying the operating conditions. Runs 1-11 had the same conditions except the residence time. In Runs 12-19 the temperature was changed to 35 ^{O}C. In Runs 20-35 ^{O}C the digester inoculum, rather than the soil inoculum, was used and some of these runs (Runs 30-35) were carried out with pre-acclimation of the inoculum. Runs 36-42 were carried out in arrange of glucose concentration in the feed and composition of mineral media. Runs 44-49 were carried out in batch with a range of glucose concentration and mineral media.

Analytical methods

For the determination of soluble species (glucose, ethanol and organic acids) reactor samples were immediately filtered (Millipore membrane of 0.45 µm) following sampling. Fermentation products were analysed by gas chromatography, Trace 1300 Thermo Scientific Series, equipped with a flame ionisation detector (FID) and a fused capillary column 30 x 0.25 TG-WaxMS A. The column temperature was 110 ^{0}C for 2 mins, 120 ^{0}C

for 3 min and 150 ^{0}C for 4 min. The temperature of the injector and detector were 200 ^{0}C and 250 ^{0}C respectively. Hydrogen was used as carrier gas. The samples were acidified with H_3PO_4 (30% v/v) and the internal standard 2-ethyl-butyric acid was used. With this method, detectable species were ethanol, acetic, propionic, butyric and caproic acids. Glucose concentration was measured using the colorimetric total carbohydrate and soluble sugars assay based on the Anthrone Reagent-Sulphuric Acid Method as described elsewhere [11]. This method uses the absorbance of coloured complexes formed between anthrone and the carbohydrates at 620 nm. The limit of detection of each analyte was 10 mg/L. VSS were determined by filtration on glass-fiber filters (Whatman, GF/C) and then heating at 105 ^{O}C and 550 ^{O}C according to the standard procedures [12].

COD balance

The COD balance [13] was calculated, as %, by adding up the COD of the detected fermentation products (organic acids and biomass) and by dividing this sum by the COD of the removed glucose. The conversion factors (g COD/g) for the main species detected were: glucose 1.067; ethanol 2.08; acetic acid 1.067; biomass (assumed to be $C_5H_7O_2N$) 1.42.

Results and discussion

Effect of residence time, inoculum source, temperature and acclimation

The residence time was the main parameter that affected glucose removal from the liquid phase (Figure 1a). Within the investigated ranges, the source of inoculum, temperature and acclimation had little effect on glucose removal. Glucose removal increased as the residence time increased, reaching over 90 %. In all the runs, ethanol and acetic acid were the only products detected at significant concentrations in the liquid phase (Figure 2), other acids (propionic and butyric) were only detected at low concentrations (corresponding to steady state yields lower than 0.01 g/g). The COD balance (Figure 1b) was in almost all runs between 85 and 110 %, indicating that most fermentation products were identified and that there was no or very little production of gas-phase products (hydrogen or methane). The only exceptions were Runs 1 and 2, were the COD balance was higher than 110 %, probably due to the very low glucose removal (which was in turn due to the short residence time) and therefore to the higher analytical uncertainty in measuring very low concentrations of products. Generally, the main process parameter that affected the observed yield was the residence time, with limited effect of the other parameters, as also observed for the glucose conversion. Comparing the yields on the fed (Figures 2a, c, e) and removed (Figures 2b, d, f) glucose, the ethanol profiles showed the same trend, while for acetic acid and biomass the profiles were somewhat different. The acetic acid yield was approximately constant with the residence time when correlated with the glucose in the feed (Figure 2c), while it was high at low residence time and then reached a minimum at intermediate values of the residence time if correlated with the removed glucose (Figure 2d). This indicated that at low residence time (up to

approximately 20 h) acetic acid rather than ethanol was the main fermentation product, however, since the glucose conversion was low, the acetic acid yield on the fed glucose was also low. The increase in the residence time caused not only an increase in glucose conversion but also a shift in the fermentation products, with an increase towards ethanol rather than acetic acid production. The biomass yield on the fed glucose increased as the residence time increased (Figure 2e) while the biomass yield on the removed glucose decreased and then reached a constant value (approximately 0.10 g/g). This indicated that a low residence time the fraction of glucose used for anabolism (assimilated into microorganisms) is higher than at higher residence times. Ethanol yield showed a maximum for intermediate values of the residence time (20-50 h, with ethanol yields on the removed glucose in the range 0.30-0.40 g/g). For these intermediate values of the residence time ethanol was the main fermentation product. At the highest residence times used in this study (96-100 h), acetic acid was again the main fermentation product, as for low residence time.

Although several studies have investigated the anaerobic acidogenic fermentation of glucose with open mixed cultures, our study extends the range of the investigated conditions. Zoetemeyer et al. [9] worked with a feed glucose concentration of 9.1 g/L in the temperature range 20-60 ^{O}C and in the range of residence times 2-14 h at pH 5.8. Depending on the process conditions, they observed ethanol, acetic, butyric or lactic acids as the main products. In our study, ethanol and acetic acid were the main products, while butyric and lactic acids were only present at very low or undetectable concentrations. One possible explanation is that our study was carried out at higher pH than the study by Zoetemeyer et al. [9] and lactic and butyric acids are generally mainly

present at acidic pH. Other differences in the medium composition and in the type of inocula used could also be reasons for the different results. Temudo et al. [8] investigated the acidogenic fermentation of glucose at a concentration of 4.0 g/L in a range of pH values from 4.0 to 8.5 at a constant residence time of either 8 or 20 h. In the pH range similar to the present study, 6.25-7.0, they found acetic acid and ethanol as the main products, in agreement with our findings. Horiuchi et al. [14] worked with glucose at 8.0 g/L in the pH range 5.0-8.0, in the residence time range 3-14 h. At pH 7, acetic and butyric acids were the main fermentation products, with lower concentrations of ethanol. Fang et al. [15] worked with glucose at 7.0 g/L, residence time 6 h and pH in the range 4.0-7.0, finding butyric and acetic acids as the main products in all conditions. Karadag and Puhakka [10] investigated the anaerobic conversion of glucose at pH 5.0, residence time 6 h, in the temperature range 37-65 ^{O}C. At 37 ^{O}C, the closest temperature to our temperature range, acetic and butyric acids were the main fermentation products.

Overall, this study greatly extends the range of investigated concentration and residence times for the anaerobic acidogenic glucose fermentation making it translatable for scaling up. The results reported in Figures 1 and 2 were obtained with glucose concentration 20 g/L, higher than any other previous studies. Furthermore, the range of investigated residence times in our study is 1-100 h, showing the feasibility of carrying out acidogenic fermentation of glucose even at residence times much longer than previously reported, with very little conversion to methane (as evident from the COD balance). Our study shows that, in the extended range considered in this study, the residence time had an important effect on product distribution, with a shift from acetic acid to ethanol. No effect was observed for the acclimation time (no acclimation vs 1 week) and for temperature (25

vs 35 ^{O}C). This indicated that, within the investigated range of the process variables considered in this study, glucose removal and the spectrum of products was mainly determined by the residence time, rather than by other factors.

Effect of glucose concentration

Glucose removal was very high for glucose concentration 5-20 g/L, but much lower, approximately 50 % in the run at 50 g/L (Figure 3a). The ethanol and acetic acid yields were also affected by the substrate concentration (Figures 3b-d). Ethanol yield showed a maximum for a glucose concentration of 20 g/L, while acetic acid yield decreased as the substrate concentration increased. It is important to observe that in the run with 50 g/L the COD recovery was only 60 %, differently from all the other runs where it was higher than 85 %. This is an indication than in this run a fraction of the glucose was converted to either other undetectable liquid phase products or to gaseous products (methane or hydrogen). In order to investigate whether the lower conversion obtained with glucose feed 50 g/L was due to the lack of any mineral elements present in the medium, several runs were carried out with different medium compositions. The supplementation of the trace element solution had the most important effect on the glucose conversion (Figure 4a), allowing to reach virtually complete conversion. Figure 4 shows the glucose conversion (4a) and the product yields (4b-d) for the different mineral media. The trace element solution gave an important increase in the ethanol yield (Figure 4b), while the acetic acid and biomass yields were virtually unaffected (Figures 4 c, d). The supplementation of the other mineral media had little effect on glucose conversion and product yield, with the exception of the increased acetic acid yield observed for the M9 medium.

The positive effect of trace elements on anaerobic digestion of organic waste to methane has been reported in a number of studies (e.g. [16-18]). However, to the best of our knowledge, this is the first time that a positive effect of trace elements has been reported on acidogenic glucose fermentation. Overall, this study shows that the composition of the mineral medium is important in acidogenic fermentation of glucose at high concentration. At lower glucose concentration, the ions in the mineral medium are enough to sustain microbial growth and product formation, however as the substrate concentration in the feed increases, a higher concentration of trace elements is required, which needs to be provided externally.

Batch experiments

Glucose was rapidly removed when the initial concentration was 5 or 10 g/L (Figures 5a, b). When the initial concentration was 20 g/L glucose was also completely removed, even though the degradation took over 30 d (Figures 5c). When the glucose concentration was 50 g/L (Figure 5d) the glucose was only partially removed. It was then decided to supplement the medium with M9 medium at day 25 and then with trace elements at day 62. The addition of M9 medium gave only limited improvement in glucose degradation, while the addition of the trace elements had a more important effect, allowing to achieve over 80 % glucose removal by the end of the experimental period. In order to confirm the effect of the trace elements, an additional batch test was carried out (glucose at 50 g/L, Figure 5e) with mineral medium and trace element solution fed from the start of the experiment. Glucose concentration decreased below 10 g/L within 30 days of the start of the test. Compared with the much slower glucose degradation observed with mineral solution only (Figure 5d), this experiment confirms the positive effect of trace elements

supplementation. Nitrogen sparging (Figure 5e, day 34) caused a rapid drop in ethanol concentration with corresponding increase in acetic acid concentration. A final batch experiment was carried out with glucose at 50 g/L with the standard mineral medium and tap water (Figure 5f), in order to see whether the mineral elements in tap water were enough to increase the glucose degradation compared to the case with deionised water only. The results with tap water were very similar to the results with the standard mineral medium (first part of Figure 5d), indicating that a more complex mineral medium than tap water was required to enhance the degradation of glucose at this high concentration.

Acetic acid was the main product at the end of the batch tests for initial concentrations up to 20 g/L (Figure 6). When the initial concentration was 50 g/L without mineral medium supplementation (with low removal of glucose) both ethanol and acetic acid yields were low. In the experiment with glucose 50 g/L and trace element supplementation, on the other hand, acetic acid and ethanol were the main fermentation products.

Batch test results had some analogies and differences with the continuous experiments. Similarly as in continuous experiments, glucose with the mineral solution (MS) was almost completely removed up to an initial concentration of 20 g/L, while when the concentration was 50 g/L the degradation was incomplete. Glucose removal when the initial concentration was 50 g/L was enhanced by the supplementation of trace elements, as already observed in continuous experiments. However, the product distribution was different in batch and continuous experiments. In the batch experiments up to 20 g/L, acetic acid was always the predominant product, while in continuous experiments both acetic acid and ethanol were the main products, the relative abundance depending on the residence time. A hypothesis to explain this difference is that acetate- and ethanol-

producing microorganisms are favoured by high and low glucose concentrations, respectively. In batch reactors the average glucose concentration during the experiments is higher than in continuous reactors, in which the substrate concentration is always at the minimum steady state level. Therefore, batch conditions favour acetate-producing microorganisms while continuous conditions favour ethanol producing ones. This is also confirmed by the higher acetate yield at low values of the residence time in continuous experiments, which had higher glucose concentration at steady state. The different product composition in batch and continuous conditions can have great importance in a biorefinery context and requires further investigation.

Another interesting evidence from batch tests is the effect of nitrogen sparging on the shift in product distribution from ethanol to acetic acid. This effect is probably due to the decrease in hydrogen partial pressure caused by sparging with an inert gas. Under anaerobic conditions the conversion of ethanol into acetic acid generates hydrogen and is thermodynamically favoured by low hydrogen partial pressure. In the absence of sparging of the head space, hydrogen partial pressure can be high (hydrogen is generated in the production of acetate from glucose) and ethanol conversion to acetate is thermodynamically inhibited. When hydrogen partial pressure is decreased due to nitrogen sparging, ethanol conversion to acetate can occur, explaining the shift from ethanol to acetate as main fermentation products. This hypothesis is consistent with other literature studies on the beneficial effect of low hydrogen pressure on high acetate yields in anaerobic fermentation [19] and can have important practical applications in the control of product distribution in biorefinery processes. For example, if ethanol is the main desired product, then the fermentation should be carried out without gas sparging, so that the

hydrogen partial pressure is maximised and the conversion of ethanol into acetic acid is minimised. On the other hand, if acetic acid rather than ethanol is the desired product, then the hydrogen partial pressure should be minimised, for example by sparging with an inert gas.

Conclusions

The residence time is the most important parameter that affects the anaerobic conversion of glucose into liquid-phase products in continuous fermentation. The residence time affects the glucose conversion (with over 80 % conversion at residence times of 30 h or higher) and the product distribution, with ethanol as main product at intermediate residence time and acetic acid as main product at low or high residence time. Biomass yield on the removed glucose is constant at approximately 0.10 g/g in a wide range of residence times. The type of inoculum used, pre-acclimation of the inoculum and the fermentation temperature (25-35 ^{O}C) had little effect on glucose conversion and on products yield. Addition of a trace elements solution had an important beneficial effect on glucose conversion at the highest concentration tested (50 g/L glucose concentration in the feed), showing that at the highest concentration glucose conversion is limited by the lack of essential trace elements. Batch fermentation experiments gave a predominance of acetic acid rather than ethanol as main fermentation product and showed that the product distribution can be manipulated by sparging with an inert gas, which favours ethanol conversion into acetic acid. In summary, under the conditions investigated in this study, optimum operating parameters for the production of ethanol from glucose are continuous fermentation at intermediate residence times (20-50 h) without gas sparging, while, if acetic acid is the desired product, batch fermentation with gas sparging should be used.

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Table 1. Experimental conditions for all the runs.

Run	Operating mode	T (°C)	Medium	Inoculum	Pre-acclimation	Glucose conc. (g/L)	Residence time (h)
1	Cont.	25	MS	Soil	No	20	1.91
2	Cont.	25	MS	Soil	No	20	2.62
3	Cont.	25	MS	Soil	No	20	6.88
4	Cont.	25	MS	Soil	No	20	10.03
5	Cont.	25	MS	Soil	No	20	16.39
6	Cont.	25	MS	Soil	No	20	19.09
7	Cont.	25	MS	Soil	No	20	24.33
8	Cont.	25	MS	Soil	No	20	28.32
9	Cont.	25	MS	Soil	No	20	37.59
10	Cont.	25	MS	Soil	No	20	48.12
11	Cont.	25	MS	Soil	No	20	96.21
12	Cont.	35	MS	Soil	No	20	1.38
13	Cont.	35	MS	Soil	No	20	3.76
14	Cont.	35	MS	Soil	No	20	6.38
15	Cont.	35	MS	Soil	No	20	10.81
16	Cont.	35	MS	Soil	No	20	20.59
17	Cont.	35	MS	Soil	No	20	25.86
18	Cont.	35	MS	Soil	No	20	36.57
19	Cont.	35	MS	Soil	No	20	100.11
20	Cont.	25	MS	Digester	No	20	1.54
21	Cont.	25	MS	Digester	No	20	3.59
22	Cont.	25	MS	Digester	No	20	5.78
23	Cont.	25	MS	Digester	No	20	10.84
24	Cont.	25	MS	Digester	No	20	16.19
25	Cont.	25	MS	Digester	No	20	20.61
26	Cont.	25	MS	Digester	No	20	24.84
27	Cont.	25	MS	Digester	No	20	31.58
28	Cont.	25	MS	Digester	No	20	38.95
29	Cont.	25	MS	Digester	No	20	50.47
30	Cont.	25	MS	Soil	Yes	20	3.46
31	Cont.	25	MS	Soil	Yes	20	6.68
32	Cont.	25	MS	Soil	Yes	20	10.93
33	Cont.	25	MS	Soil	Yes	20	25.82
34	Cont.	25	MS	Soil	Yes	20	32.64
35	Cont.	25	MS	Soil	Yes	20	36.75
36	Cont.	25	MS	Soil	No	5	24.13
37	Cont.	25	MS	Soil	No	10	24.08
38	Cont.	25	MS	Soil	No	20	24.24
39	Cont.	25	MS	Soil	No	50	24.39
40	Cont.	25	MS	Soil	No	50	96.41
41	Cont.	25	MS+M9	Soil	No	50	96.67
42	Cont.	25	MS+TE	Soil	No	50	96.45
42	Cont.	25	MS tap	Soil	No	50	96.11
44	Batch	25	MS	Soil	No	5	-
45	Batch	25	MS	Soil	No	10	-
46	Batch	25	MS	Soil	No	20	-
47*	Batch	25	MS	Soil	No	50	-
48**	Batch	25	MS+TE	Soil	No	50	-
49	Batch	25	MS tap	Soil	No	50	-

* In run 47 the medium composition was changed to MS+M9 (day 27) and then to MS+M9+TE (day 62) during the run; ** In run 48 the reactor was sparged with nitrogen at day 34.

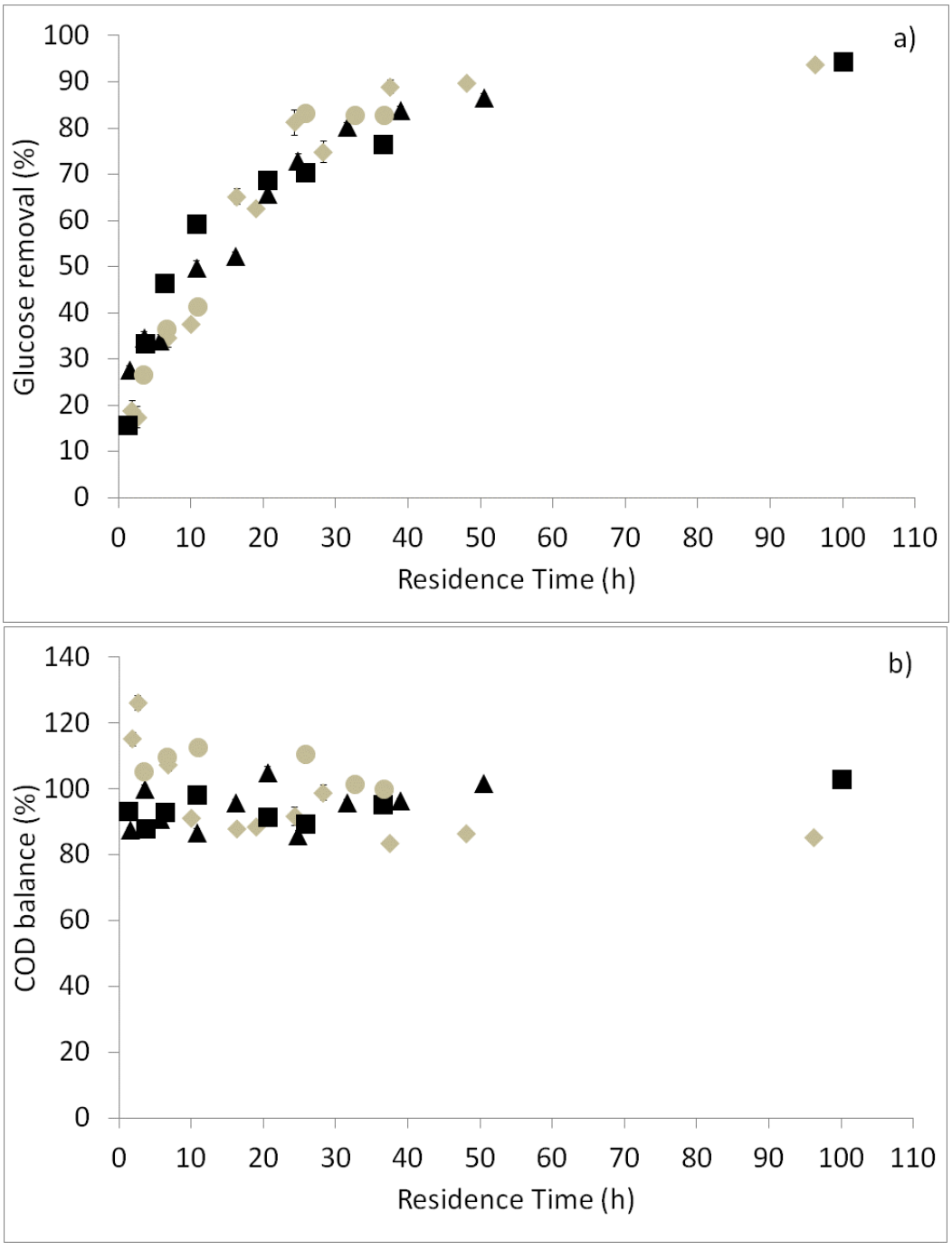

Figure 1. Glucose conversion at steady state a) and COD balance b) in continuous runs. ◆ = Runs 1-11; ■ = Runs 12-19; ▲ = Runs 20-29; ● = Runs 30-35. Error bars indicate the standard deviation (when not visible they are within the size of the data point).

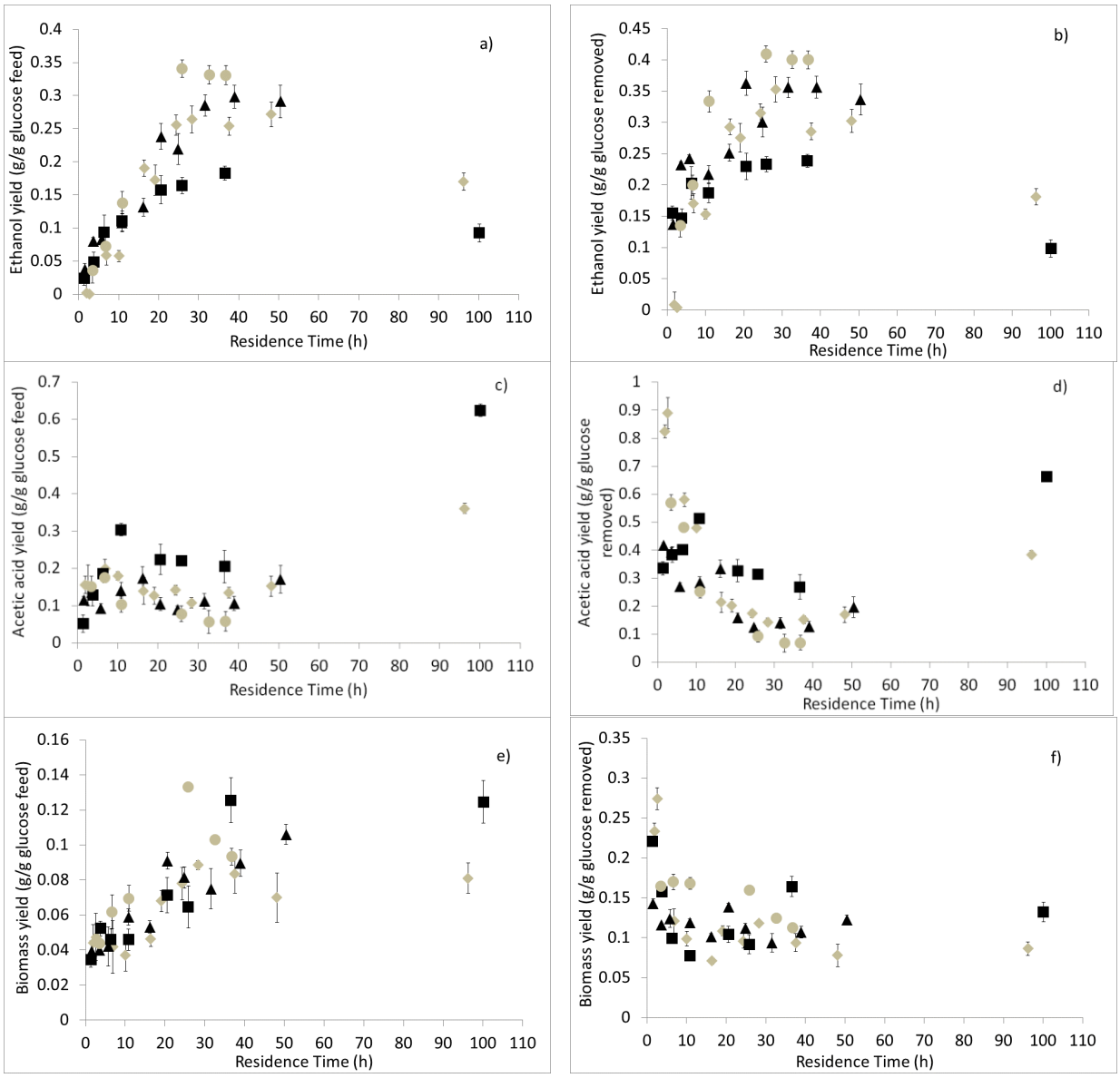

Figure 2. Ethanol, acetic acid and biomass yields in continuous runs. a), c), e): yields on the glucose in the feed; b), d), f): yields on the removed glucose. ◆ = Runs 1-11; ■ = Runs 12-19; ▲ = Runs 20-29; ● = Runs 30-35. Error bar indicate the standard deviation.

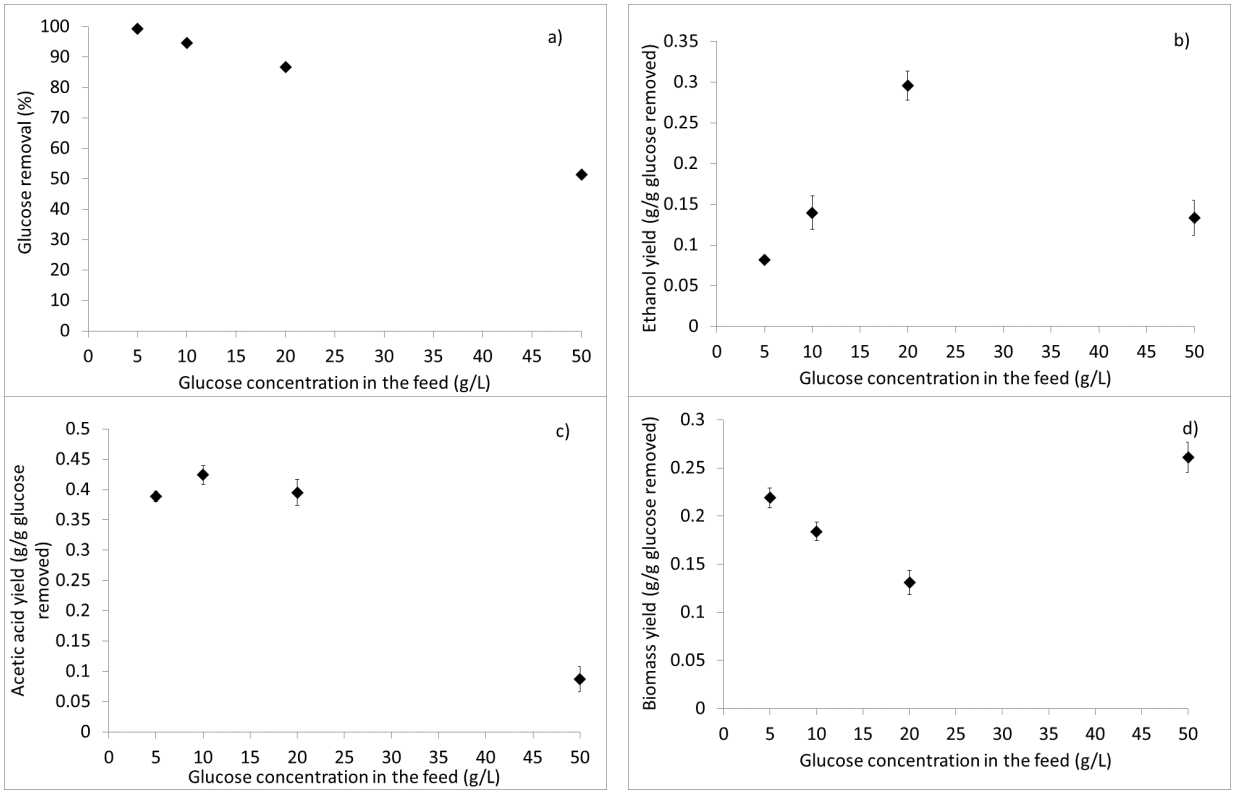

Figure 3. Continuous runs with glucose at different concentrations (Runs 36-39). 3a: glucose conversion; 3b,c,d: ethanol, acetic acid and biomass yields. Error bars indicate the standard deviation.

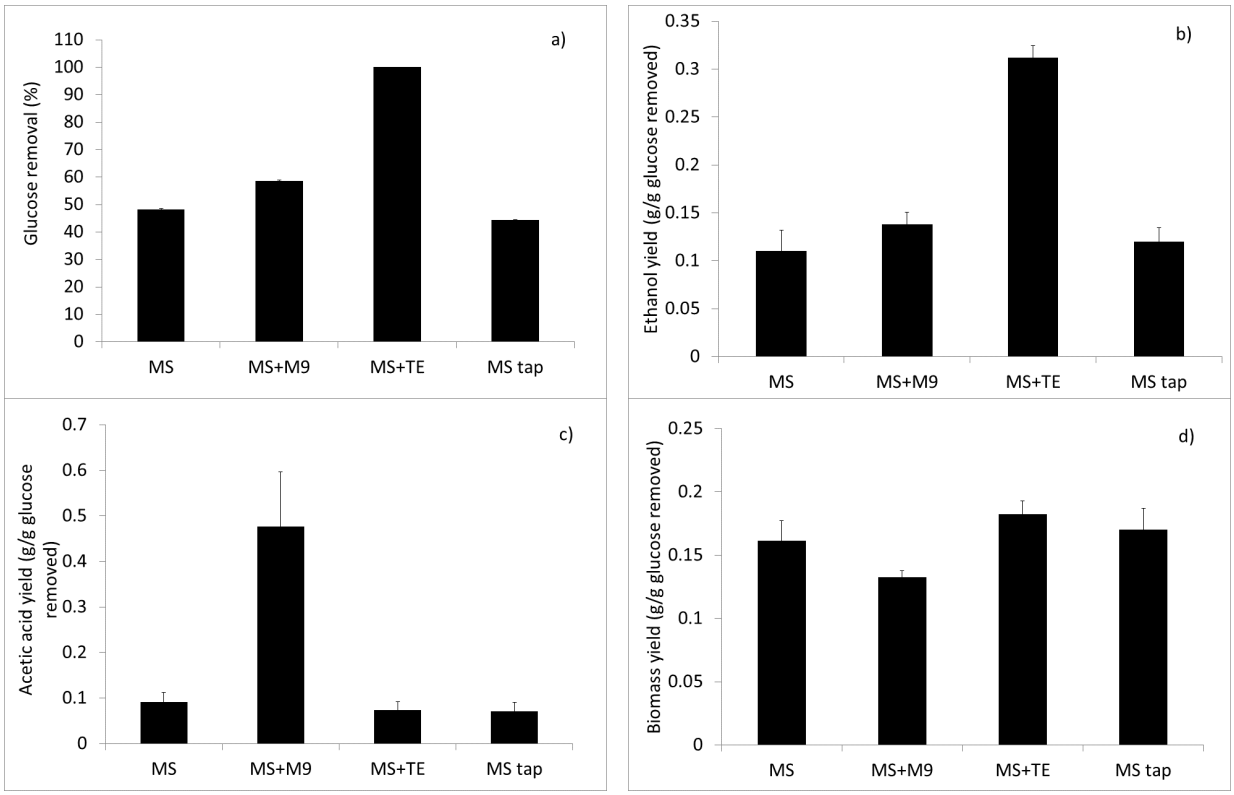

Figure 4. Effect of medium supplementation on glucose conversion (4a) and product yields on the removed glucose (4b, c, d), runs 39-42. Error bar indicate the standard deviation.

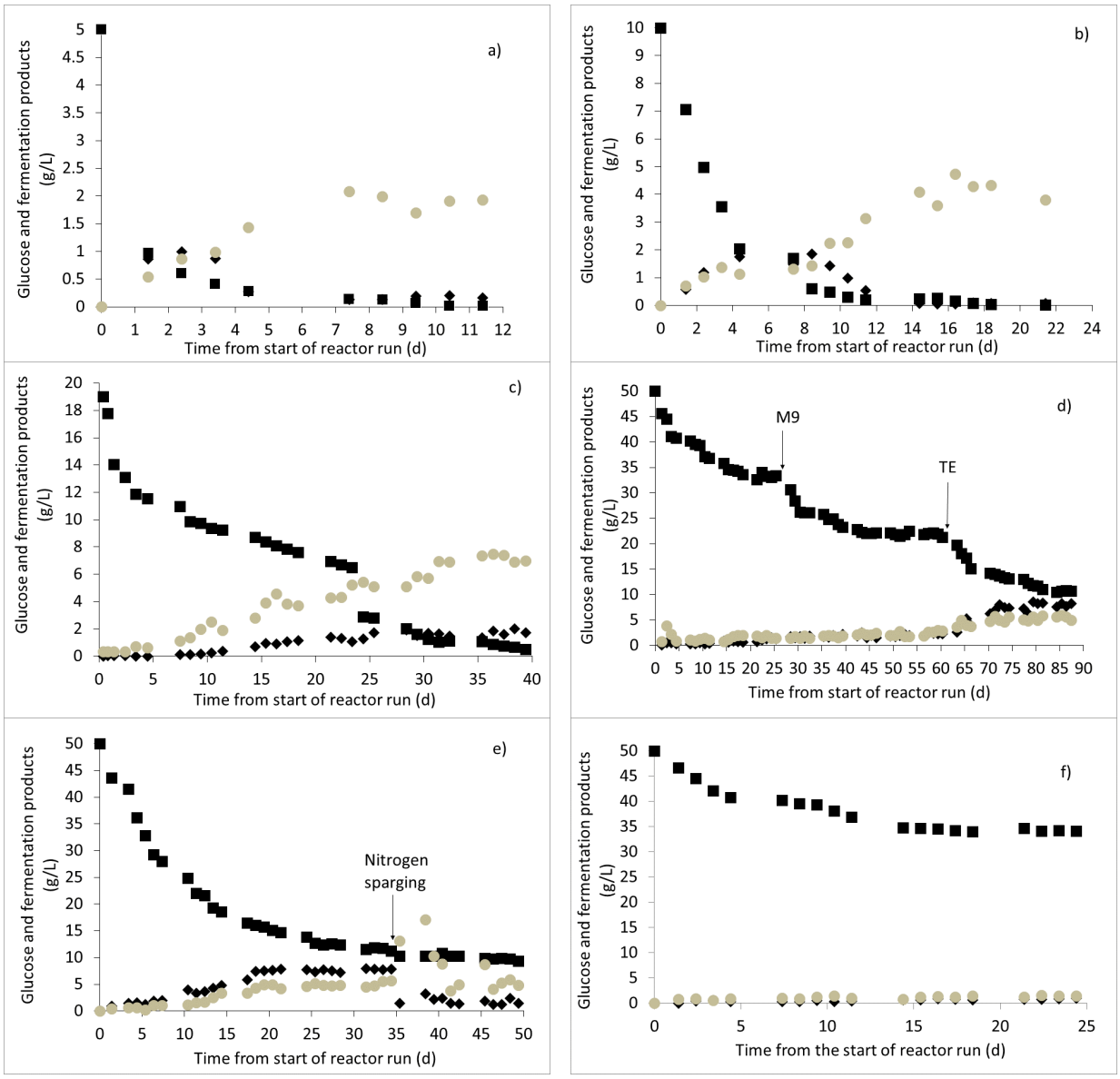

Figure 5. Batch tests at different glucose concentrations, runs 44 (a), 45 (b), 46 (c), 47 (d), 48 (e), 49 (f). ■ = glucose; ● = acetate. ◆ = ethanol.

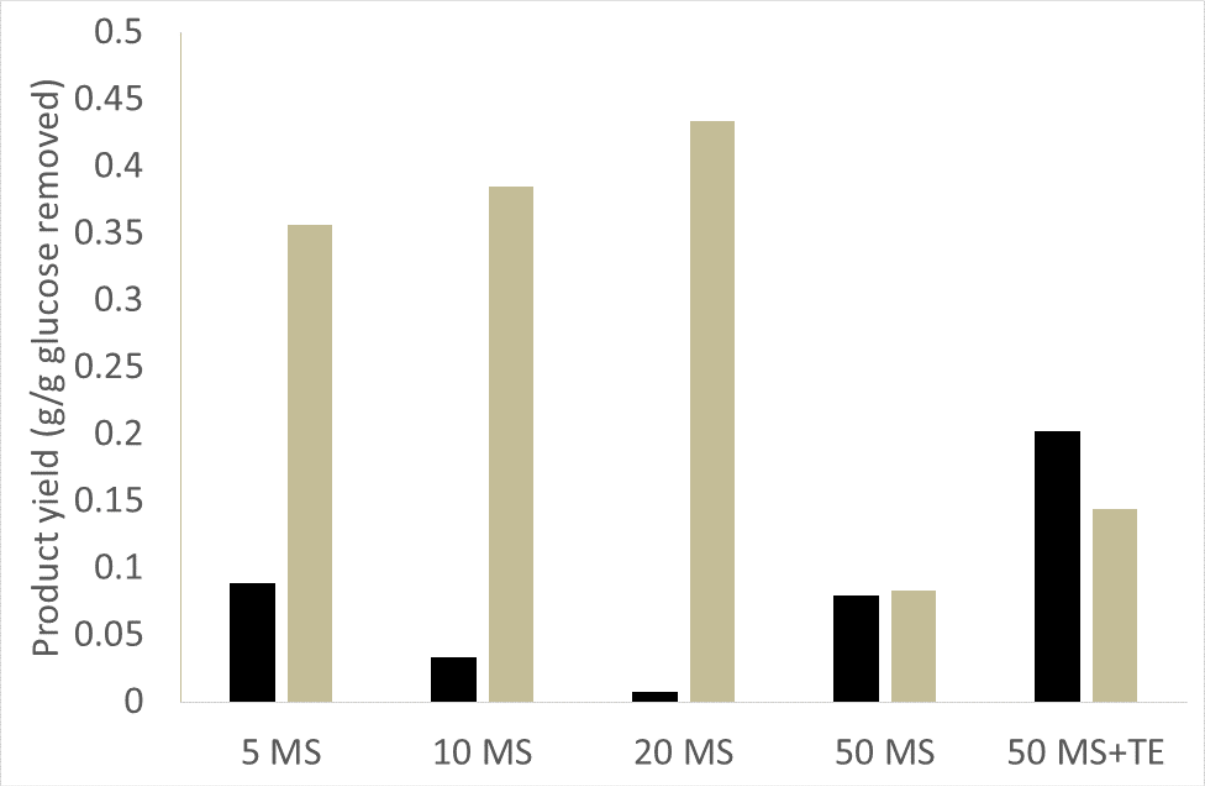

Figure 6. Product yields in batch tests (runs 44-49), the notation on the horizontal axis refers to the glucose concentration at the start of the experiment and to the composition of the mineral medium. ■ = ethanol; ■ = acetate.