

Anaerobic fermentation for the production of short chain organic acids: product concentration, yield and productivity in batch experiments at high feed concentration

Serena Simonetti^a, Claudia Fernández Martín^{a,b}, Davide Dionisi^{a*}

^aSchool of Engineering, Materials and Chemical Engineering Group, University of Aberdeen, Aberdeen AB24 3UE, UK

^bCentre for Energy Transition, University of Aberdeen

s.simonetti.18@abdn.ac.uk, cfmartin@abdn.ac.uk, davidedionisi@abdn.ac.uk

*corresponding author: davidedionisi@abdn.ac.uk

Abstract

With the general aim of maximising product concentration, yield and productivity in the anaerobic fermentation (AF) of biomass to make short-chain organic acids (SCOAs) with low-cost and environmentally sustainable processes, this study investigates the effect of high substrate concentration on the batch digestion of model biomass at uncontrolled temperature. Five substrate concentrations, between 24.7 and 394.6 gCOD l⁻¹ (the latter is the highest substrate concentration reported in the literature for these types of studies), were investigated in batch reactors with no pH control. The highest substrate concentration led to a maximum product concentration of 61.5 g l⁻¹, composed mainly of lactic acid (85 wt%). The lowest substrate concentration produced mainly acetic acid (54 wt%). The pH was acidic in all cases but was higher for the most diluted feed (6.2). Similar yields at the end of the experiments, between 15.7 and 22.0 % COD COD⁻¹, were observed in all reactors. Over the length of the experiments, generally yields increased and productivities decreased, indicating the need of a compromise between yield and productivity. For the highest substrate concentration, a final productivity of 1.5 g l⁻¹ d⁻¹ was obtained.

Experiments with pH adjustment in the range 4.0-6.0 were performed, and results generally showed an increase in product concentration, yield and productivity as the target pH increased, with the highest values of concentration, yield and productivity (163 g l⁻¹, 56 % COD COD⁻¹ and 3.9 g l⁻¹ d⁻¹ respectively) obtained at pH 5.5.

Keywords: anaerobic fermentation (AF); volatile fatty acids (VFAs); short chain organic acids (SCOAs); organic waste

Abbreviations

Anaerobic fermentation (AF); chemical oxygen demand (COD); insoluble carbohydrates (IC); soluble carbohydrates (SC); short chain organic acids (SCOAs); soluble chemical oxygen demand (SCOD); total carbohydrates (TC); total chemical oxygen demand (TCOD); total solids (TS); total suspended solids (TSS); volatile fatty acids (VFAs); volatile solids (VS); volatile suspended solids (VSS).

1 Introduction

Due to the need of using renewable feedstocks as carbon source, valorisation of waste and biomass for the production of bulk chemicals is becoming of growing interest. Anaerobic digestion, a natural process where microorganisms degrade organic matter in the absence of air, can be tailored for this purpose. As an industrial established technology, anaerobic digestion has been so far exploited to obtain biogas and digestate, used as heat and power source and as biofertilizer. Nevertheless, in the intermediate steps of the process, called acidogenesis and acetogenesis, the hydrolysed organic matter is degraded into short-chain organic acids (SCOAs, e.g. lactic acid, acetic acid, propionic acid, butyric acid) and other byproducts such as alcohols, ammonia and hydrogen [1]. When the process is aimed at the production of SCOAs and other intermediates, rather than methane, it is more commonly referred to as anaerobic fermentation (AF), which is the terminology we will use in this study. It has been calculated that, with the annual global generation of organic waste, SCOAs could be produced via AF at rates much higher than their current production [2]. SCOAs have higher economic value than methane, because of their various applications as marketable chemicals or as precursors (i.e. applications in the cosmetic, food, pharmaceutical and chemical industry). SCOAs can be used as a carbon source for nitrogen and phosphorus removal from wastewater [2], in microbial fuel cells to produce electricity [3], and as a substrate for biopolymer synthesis [4]. Moreover, carboxylates can be converted to esters, carbonyls, alkanes or alcohols by using chemical or microbial routes [5]. Currently, SCOAs are produced from non-renewable resources (natural gas) or pure microbial cultures fed on organic substrates (whey, molasses, sugarcane, starch), which can compete with the food market [6-11]. Furthermore, the production of SCOAs from non-renewable resources is often based on the use of metal catalysts, which are also non-renewable, and is carried out at high temperatures (150–200 °C for acetic acid and 70–150 °C for butyric acid), with associated energy consumption and potential process safety issues [6]. The main advantages of the production of SCOAs from organic waste via AF are the use of a renewable resource with milder reaction conditions, without metal catalysts, and the provision of a route for waste valorisation.

In spite of these advantages, SCOAs production via AF has not reached commercial scale yet. The main drawbacks of SCOAs production via AF are the need to inhibit methanogenesis, the relatively low concentration of the produced SCOAs, which implies high separation costs, and the relatively low volumetric productivity of the process.[12] Therefore, in order to make the production of SCOAs via AF economically competitive and environmentally sustainable, process performance parameters such as product concentration, yield and productivity need to be maximized through the optimization of process conditions [6, 13]. Among several parameters, substrate concentration in the feed is expected to have a strong effect on product concentration and productivity. However, to the authors' knowledge, few experiments which investigated high substrate concentrations have been performed. Our recent literature review [14] showed that, in studies on acidogenic fermentation to produce SCOAs, the mean substrate concentration was 53 gCOD l⁻¹, and only 10 % of the studies had a substrate concentration higher than 128 gCOD l⁻¹. Consequently, the mean product concentration was lower than 10 g l⁻¹ and only 10 % of the studies reported product concentrations higher than 23 g l⁻¹ [14]. These product concentrations are usually too low for commercial exploitation of this process. Moreover, the evaluation of the effect of the initial substrate concentration is rarely assessed, unlike other parameters like pH, temperature and organic loading rate [15]. In spite of the limited literature studies on anaerobic SCOAs production with concentrated feed, there are many potential anaerobic digestion feedstocks with high concentration of organic matter. For example, the COD of undiluted food waste has been measured as 286 g/kg [16], poultry and livestock manure can have more than 20 % solids [17], and the solids content of sugar beet, grass and willow at harvest was measured in the range 20-50 % [18].

When compared to waste feedstocks which are currently used for anaerobic digestion, food waste is a prime substrate due to its high moisture content, balanced macronutrients composition and C/N ratio [19]. Among treatment technologies for food waste disposal, anaerobic digestion has also the lowest impacts on climate change and greenhouse gas emissions [20-22]. On one hand, food waste production has economic and environmental consequences, it exploits natural resources (e.g., land, water) and impacts biodiversity [23]. On the other hand, food waste is generated at high enough amounts to replace current feedstocks used to

produce SCOAs. Worldwide, 1.3 billion tonnes per year of food are lost or wasted [24], whereas in the UK 8.3 million tonnes per year of household food and drink are wasted [25].

In this work, batch experiments have been performed for the investigation of different initial substrate concentrations of a model organic waste, with the aim of maximising the key performance variables namely product concentration, yield and productivity. In order to have a process that is potentially economically attractive and not energy intensive (although the economic and energy analysis was not the aim of this study), the experiments were carried out without pH control, which avoids the addition of external chemicals, and at room temperature, to minimise energy consumption. For comparison, some experiments were also carried out with pH adjustment in the range 4.0-6.0. To the best of our knowledge, the highest substrate concentration used in this study (395 gCOD l⁻¹) is the highest feed concentration reported in acidogenic fermentation studies to produce SCOAs. Our study gives therefore an insight into the maximum SCOA concentration that is possible to obtain in acidogenic fermentation processes.

2 Materials and methods

2.1 Materials

To simulate food waste, the following chemicals were used: organic wheatgrass 100 %, powder (Naturya); yeast extract 100 % (Fisher BioReagents™); starch 100 %, soluble (ACROS Organics™); peptone 100 %, powder (Fisher BioReagents™); d-sucrose 100 % (Fisher BioReagents™); oleic acid, 90 % (Alfa Aesar™). For COD analysis, COD cell tests photometric, 5000-90000 mg/L (COD) (Spectroquant®), were used. To analyse carbohydrates, anthrone, ACS reagent, 97 % (Sigma-Aldrich) and sulfuric acid, SLR, min 95% (Fisher Chemical™) were used. To prepare the internal standard for SCOAs analysis, 2-Ethylbutyric acid, 99 % (Sigma-Aldrich), and orthophosphoric acid, 85+ % (Fisher Chemical™), were used. SCOAs used for calibrations were: lactic acid, extra pure, SLR (Fisher Chemical™); ethanol, 99%+, absolute, extra pure, SLR (Fisher Chemical™); sodium acetate anhydrous, 99 % (Fisher Chemical™); sodium propionate, 99 % (Fisher Chemical™); isobutyric acid, 99+ % (Fisher Chemical™); sodium butyrate, 99 % (Fisher Chemical™); isovaleric acid, 99 % (ACROS Organics™); valeric acid, 99 % (ACROS Organics™); 4-methylvaleric acid, 99 % (ACROS Organics™); hexanoic acid, 99 % (ACROS Organics™). To prepare buffer solution, sodium hydroxide, white pellets (Fisher BioReagents™), was used.

2.2 Substrate and inoculum

Five different initial substrate concentrations (A, B, C, D, E, from the most to the least concentrated) were tested in batch experiments. The dilution factor from one concentration to another was 2. The components used to prepare the model undiluted substrate are displayed in Table 1, and they were chosen to simulate the household food and drink waste in the UK in terms of macronutrients composition (carbohydrates, fibers, sugars, proteins and lipids) and concentration [26]. Substrate characterisation is shown in Table 2. The inoculum was an anaerobic mesophilic sludge collected at the bottom of the anaerobic reactor of GaskFarm in Turriff (Scotland). The digester is a 2500 m³ tank which treats bakery, fish and cow's waste at 40 °C and 50 days (retention time). The inoculum was a black viscous sludge that was stored at 4 °C after collection. Prior

to use and analysis, inoculum was filtered using a Buchner funnel to remove large solids. Table 3 shows the characterisation of the inoculum.

2.3 Experiments set-up

Five hermetically closed glass reactors (named A, B, C, D, E) had a working volume of 300 ml and were operated for 42 days in batch mode in four replicates. Three of these replicates included sampling at regular intervals during the run, one replicate only included sampling at the end of the run. Prior to start, reactors were filled with substrate and flushed with nitrogen for 10 minutes. Then, 5vol% of inoculum was added. The reactors were magnetically stirred (300-450 rpm), were operated at ambient temperature (approximately 25 °C) and without pH control. A long-term batch experiment, run for 168 days and sampled once a month, was performed in duplicate in two identical 1l reactors with substrate concentration A.

Some experiments were carried out with pH adjustment, without replicates. Four 300 ml reactors with substrate concentration A (named A4, A5, A5.5, A6) were set up similarly to the other batch experiments and run for 42 days. In these reactors the pH was adjusted manually, several times per day during working days, by adding a solution of NaOH 5M to bring the pH up to the desired value (4.0, 5.0, 5.5 or 6.0).

2.4 Analytical methods

The total and volatile suspended solids (TSS and VSS) and total and volatile solids (TS and VS) were determined following the APHA-AWWA-WPCF 1992 procedure [27]. Fermentation products considered for the calculation of the process parameters were SCOAs (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid, lactic acid) and ethanol. The aforementioned liquid products were analysed following the method described by Raposo *et al.* [28]. A gas chromatograph (GC) Trace 1330, from Thermo Scientific, was used for the analysis, after filtration of the sample on a glass microfibre filter grade GF/F, porosity 0.6-0.8 µm. A solution of 2-ethylbutyric acid in 30 vol% phosphoric acid (6 g l⁻¹) was prepared and used both as internal standard and to acidify the samples. The filtered sample (1 ml) was placed in a glass vial, to which 200 µl of internal standard were added. The acidified samples were analysed in a GC equipped with a flame ionisation detector (FID) in split mode with a split injector and a TG-

Wax MS A capillary column (length: 30 m, I.D.: 0.25 mm, thickness: 0.5 μm). The initial temperature of the column (80 $^{\circ}\text{C}$) was held for 2 min, followed by a temperature increase to 200 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C min}^{-1}$, and held for an additional minute. Injector and detector temperatures were kept at 250 $^{\circ}\text{C}$. Hydrogen and synthetic air were used as carrier gas at flowrates of 35 ml min^{-1} and 350 ml min^{-1} , respectively. The total column flowrate was 1.2 ml min^{-1} and the split flowrate was set at 24 ml min^{-1} . Calibration was made by preparing a standard solution of ethanol, acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric acid, n-valeric acid, isocaproic acid and n-caproic acid, each at a concentration of 5 g l^{-1} . Lactic acid standard solution was prepared separately at a concentration of 20 g l^{-1} . Total and soluble chemical oxygen demand (TCOD and SCOD) were measured with COD kits (Spectroquant COD cell test, from Merck Millipore), which contain potassium dichromate, silver sulphate as catalyst, sulphuric acid and mercury sulphide. The kit used was in the range of 5,000-90,000 mg l^{-1} , therefore samples were diluted to be in the range. The TCOD and SCOD measuring method corresponds to DIN ISO 15705 and is equivalent to APHA 5220 D. A spectrophotometer (Thermoreactor Spectroquant $^{\circ}$ TR 620) was used to process the samples at 148 $^{\circ}\text{C}$ and the concentration was read in a photometer (Spectroquant $^{\circ}$ NOVA 60 A) pre-calibrated for these specific kits via integrated bar-code. The pH was measured by using the electrode (P12/BNC probe, Sentek), connected to a pH meter (from Mettler Toledo). Anthrone was used as a colorimetric method for total and soluble carbohydrates (TC and SC) analysis, according to Koehler's method [29].

2.5 Calculations

For SCOA's yield calculation, products concentrations were reported in terms of gCOD l^{-1} . Total products yield, productivity, and TCOD, VSS, TC and SC removal were calculated using Eq. 1 to 4 as follows:

$$\text{Yield (\%)} = \frac{\text{gCOD l}^{-1} \text{ products}}{\text{gCOD l}^{-1} \text{ feed}} \cdot 100 \quad \text{Eq 1}$$

$$\text{Productivity (g l}^{-1} \text{d}^{-1}) = \frac{\text{g total products}}{Vr \cdot \text{time}} \quad \text{Eq 2}$$

$$\text{X removal (\%)} = \frac{X_{in} - X_{end}}{X_{in}} \cdot 100 \quad \text{Eq 3}$$

Where V_r is the reactor volume, X is the concentration of the parameter of interest (TCOD, VSS, TC, SC), X_{in} is the parameter value at the start of the run, and X_{end} is the parameter value at the end of the run. Insoluble carbohydrates (IC) were calculated from the difference between TC and SC.

X_{end} was measured, while X_{in} was calculated as the sum of the X measured in the feed and in the inoculum:

$$X_{in} = 0.95 * X_{feed} + 0.05 * X_{inoculum} \quad \text{Eq 4}$$

3 Results

Figure 1 shows the profiles of product concentration, yield, productivity and pH over time. Product concentration (Figure 1a) generally increased with time, reaching 24.7, 20.6, 9.3, 5.1 and 3.0 g l⁻¹ in reactors A, B, C, D, and E, respectively, after one week. Product yields (Fig. 1b) increased with time, they were in the range of 5 to 15 % COD COD⁻¹ in day 7 and in the range of 15 to 22 % COD COD⁻¹ in the final sampling time (day 42). Reactor A had the lowest yield in the initial stage of the run but it reached a final yield in the same range as the other reactors. The experiments were all carried out with the same length (42 d) to ensure that the maximum production of SCOAs was reached, without observing any large production of methane, as analysed later in the Discussion section. Experiments carried out for longer time with feed A showed no further increase in yield and product concentration (data shown in Supplementary Information, Figure S1 and S2), which indicates that 42 days was enough to reach the maximum product concentration and yield under these experimental conditions. Fig. 1c shows that productivity was higher in the initial phase of the experiments and then decreased and that productivity was higher for the most concentrated feeds (as observed for the product concentration), with observed maximum productivity of 5.1 g l⁻¹ d⁻¹ for reactor A after 1 day of run. The pH (Fig. 1d) dropped in the first week from values close to neutrality to around 4. After day 7, the pH remained approximately constant with time in all reactors, except for reactor E in which the pH increased up to close to neutrality. Relationships between initial substrate concentrations and process performance variables measured at the end of the runs can be observed in Fig. 2. A linear correlation between product concentration measured at the end of the run and initial substrate concentration can be observed in Fig. 2a. The highest product concentration at the end of the run was achieved in reactor A (61.5 g l⁻¹), followed by reactor B (35.6 g l⁻¹). The lowest product concentration was found in reactor E (3.1 g l⁻¹), while reactors C and D reached 18.2 and 7.4 g l⁻¹, respectively. The final product yield (Figure 2b) was independent of the initial substrate concentration, being in the range of 15.7 to 22.0 % COD COD⁻¹. The final productivity (Figure 2c) showed a similar trend as the one observed for final product concentration. Final productivity increased linearly with increase in initial substrate concentration. The highest productivity at day 42 was

observed in reactor A ($1.5 \text{ g l}^{-1} \text{ d}^{-1}$). The final productivities in reactors B, C, D and E were 0.85, 0.43, 0.18 and $0.07 \text{ g l}^{-1} \text{ d}^{-1}$, respectively.

COD removal at the final sampling time (Figure 3a) was virtually zero in all runs except for the lowest initial substrate concentration (19.1 %, reactor E). The final VSS removal (Figure 3b) decreased as the initial substrate concentration increased. The highest value was observed for reactor E (42.6 %) and the lowest value for reactor A (0.8 %). A similar trend was observed for carbohydrates removal (Fig. 4a, b, c), which decreased as the initial substrate concentration increased. The 86.1 % of total carbohydrates were removed in reactor E, 79.4 % in reactor D, 73.7 % in C, 44.6% in B and 36.7 % in A. Soluble carbohydrates were almost fully removed in reactors C, D and E. (94.9, 93.6 and 97.4 % removal, respectively). The highest removal of insoluble carbohydrates achieved was 67.4 % for reactor E, while the 28.6 % removal was achieved with the most concentrated substrate (reactor A).

Figure 5 shows the final product composition and pH of reactors A, B, C, D and E. Reactors A and B not only had the highest product concentrations and productivities, but also the higher lactic acid percentage produced at the end of the runs. Lactic acid constituted over 80 wt% of all products (namely 85 and 89 wt% in reactors A and B respectively). Lactic acid percentage decreased as the initial substrate concentration decreased in the other reactors, while acetic acid percentage increased up to 54 wt% in reactor E. Propionic acid and butyric acid were also produced in a similar amount in reactor E (10 and 8 wt%), which also had the highest final pH value (6.2).

Figure 6 shows the experiments with pH adjustment and, for comparison, without pH control (reactor A). Figure 6a shows the pH value over time. Some variation in the pH was observed in the first two weeks due to the manual adjustment. After the first two weeks, the pH remained relatively constant at the desired value. Figures 6b-d show the final product concentration, yield and productivity as a function of the final pH. Product concentration, yield and productivity were higher in the experiments with adjusted pH than in the experiments without pH control. At pH 5.5 (reactor A5.5), which gave the best results, the product concentration, yield and productivity were 163.4 g l^{-1} , 56.3 % COD COD⁻¹ and $3.89 \text{ g l}^{-1} \text{ d}^{-1}$, respectively. Figure

6e shows the final product distribution and final pH value. Lactic acid was the main product at all pH (85-87 wt%), followed by acetic acid (5-9 wt%).

4 Discussion

An important evidence from the experimental data is that the total final product yield for the most concentrated feed was in the same range as for the more diluted feeds. This indicates that, under the investigated conditions, it is possible to work with highly concentrated feed without any negative effect on the product yield. This is important for the commercial scale production of organic waste from biomass since high feed concentration maximises product concentration and productivity. Although the final yield obtained (15.7-22.0 % COD COD⁻¹) indicated that the conversion of the feed COD into the desired products was only partial, it is worth noting that our recent study [30] indicated that the conversion via AF of a very little fraction of the organic waste generated globally could potentially satisfy the global demand for organic acids. Therefore, a relatively low yield of organic acids in an AF process is not necessarily a problem, if more value can be obtained from the unconverted waste, e.g., via conversion to energy in a methanogenic digester (at more neutral pH). Clearly, for the commercial success of the considered process, a high product concentration is also important, and the highest total concentration obtained in this study (61.5 g l⁻¹) is among the highest values reported for acidogenic conversion processes [14]. To the authors' knowledge, batch experiments on AF of organic substrates investigated lower substrate concentrations than the ones chosen in this study. The highest substrate concentration (191 gCOD l⁻¹) in batch experiments was studied by Jiang *et al.* [31], who also investigated different temperatures and pH values. Product concentrations (up to 48.9 g l⁻¹), considered as the sum of SCOAs and ethanol, were lower than those obtained in our study, while the yield (37.6% COD COD⁻¹) was higher, and the best results were obtained at 35 °C and pH 6. At pH 3, 5 and 7, lower yield of SCOAs was observed. Ma *et al.* [32] reported their highest SCOA concentration (53.87 g l⁻¹) at pH 6, by fermenting 141.7 gCOD l⁻¹ of food waste. Wang *et al.* [33] investigated different pH values with a food waste concentration of 162.6 gCOD l⁻¹: the highest SCOA concentration and yield were achieved at pH 6 (51.3 gCOD l⁻¹ and 918 mg/g VSS_{removed}). Lactic acid was analysed separately and produced at higher concentrations at pH 4 and uncontrolled pH (3.5): 18.50 g l⁻¹ and 14.62 g l⁻¹ respectively, after 20 days. Our experiments with pH control also showed that pH 6 and 5.5 led to higher product concentrations (121.9 and 163.4 g l⁻¹, respectively), compared to pH 5 and 4 (107.5 and 51.6 g l⁻¹, respectively). Slezak *et al.* [15]

investigated a substrate range between 4.1 and 48.2 gVS l⁻¹, observing a positive correlation between initial substrate concentration and VFAs concentration, which reached 9.81 g l⁻¹ at the highest concentration. Arslan *et al.* [34] studied different initial concentrations of potato processing waste streams with no pH control and different headspace conditions. In their study, the maximum product concentration of 15 gCOD l⁻¹ of SCOAs was reached with the most concentrated substrate (23 gCOD l⁻¹). However, higher yields (up to 95 % COD COD⁻¹) were favoured by substrate dilution, differently from our study. Studies which evaluated lactic acid production reported acidic pH as suitable for lactic acid production. Tang and Herrero-Garcia [35, 36] observed highest lactic acid production at pH 5 and 5.5, compared to uncontrolled pH (3.5 and 3.6 respectively), reaching concentrations of 28.4 and 23.4 g l⁻¹ respectively. These results are in accordance with the experiments performed with pH control, where pH 5.5 and 5 led to higher lactic acid production (142.1 g l⁻¹ and 93.0 g l⁻¹ respectively) and pH 4 led to lowest concentrations. However, in our study pH 6 was also investigated and 106.2 g l⁻¹ of lactic acid were produced after 42 days of run.

The time profiles of yield and productivity obtained in the experiments with no pH control indicate the need for a compromise between these two performance variables, at least for a batch process such as the one considered here. Indeed, the highest productivities are obtained for short run times, where the yield is the lowest (Fig. 1b and 1c). However, there is relatively little change in the productivities from day 14 to day 42, while the yield generally increases in this time range, indicating that it is probably worth running the process for the full length of the experimental period investigated in this study. This is also evident from Figure 7, which shows the productivity vs yield for the experiments in Figure 1. The highest productivity coincides with the lowest yield, however as the yield increases the decrease in productivity is less steep showing that further increases in yield don't cause much detriment to the productivity, at least under the experimental conditions investigated here.

The fact that COD removal was virtually zero in runs A-D indicates, from the COD balance [37], that in these runs there was virtually no production of methane or hydrogen. This is desirable from the point of view of maximizing production of organic acids, rather than gaseous products. The modest COD removal observed in reactor E (lowest initial substrate concentration) is probably caused by the smaller feed concentration,

which in turn gives lower concentration of acids and higher pH, which are conditions more favorable for methanogenesis [38].

Solids removal (expressed as VSS), when related to the obtained pH, had a similar trend as the observed by Tang *et al.* [39], where TSS removal was higher at pH 5 and 6 (30-50 %) compared to pH 4 and uncontrolled (3.5). Tang *et al.* also obtained similar results to ours on carbohydrates removal, observing a faster degradation of carbohydrates and soluble carbohydrates at pH of 5 and 6. In that study, SC constituted 1.5 and 0.5 % respectively at the end of the experiment, while higher percentages of 11.9 and 17.0 % were observed with respectively uncontrolled pH and pH 4. In the study of Wang *et al.* [33], carbohydrates were also totally consumed by the end of the experiment at pH 5 and 6, differently than at pH 4 and uncontrolled. Therefore, pH, rather than substrate concentration, seems to be the factor which influences solids and carbohydrates removal. The fact that in our study the removal of VSS and carbohydrates generally decreased as the initial substrate concentration increased without a corresponding effect on the product yield, might indicate that other products, unidentified and different from those analytically determined in this study, were formed in the more diluted feeds [40]. However, this deserves further study.

The composition of the products is also important for the process performance. The fact that the most concentrated feeds gave mostly lactic acid is positive if lactic acid is the desired product. Lactic acid is of growing importance to produce biodegradable plastics and this study indicates a possible sustainable process for its production. More dilute feeds gave a wider range of products, which can be favorable if other products, e.g., acetic acid, are desired. Generally, however, the conversion of the feedstock into mainly one product has to be seen favorably because of the likely reduction in separation costs. Therefore, this study is particularly interesting for the production of lactic acid from concentrated feedstocks.

The experiments with pH adjustment indicated that the product concentration, yield and productivity can be significantly increased by controlling the pH to a less acidic value. The maximum values of the product concentration, yield and productivity, obtained at pH 5.5, were amongst the highest reported in the literature [14]. However, in the practical implementation of the process, it needs to be seen whether the better process

performance outweighs the cost of adding the chemicals for pH control. An important evidence from the experiments with pH adjustment, ran with the most concentrated feed, is that the product composition is very similar in all cases, with a predominance of lactic acid. Analysing the product composition in the experiments with adjusted pH (Figure 6e) and in the experiments without any pH control (Figure 5), there is an indication that both the concentration of the feed and the pH play a role in determining the prevalence of lactic or acetic acid. Indeed, when the feed is very concentrated, lactic acid is always the predominant product in all the pH range (4-6) considered in this study. However, if pH reaches approximately 6 with the most diluted feed (feed E, Figure 1d), then acetic acid is the main product. A possible explanation of this result is that lactic acid is more acidic than acetic acid and therefore it is more dissociated at any pH values. It is reported in the literature that the toxicity of short chain organic acids on anaerobic microorganisms is mainly due to the undissociated form rather than to the dissociated form [41]. Using literature values of 3.86 and 4.76 for the pKa of lactic and acetic acid, respectively, it can be calculated that, at pH 4.0, the fraction of the acid that is undissociated is 42 % for lactic acid and 85 % for acetic acid. At pH 6.0, the fraction of the undissociated acid is 0.7 % for lactic acid and 5.2 % for acetic acid. When the concentration of the feed is high, the production of acetic acid would result in high concentrations of undissociated species in all the pH range (4-6) considered in this study. Therefore, with concentrated feed the fermentation always shifts towards the production of lactic acid. When the concentration of the feed is lower (such as feed E in our study), the fermentation can produce acetic acid rather than lactic acid because of the lower concentration of products and therefore the lower risk of inhibition due to the undissociated acids. However, further investigation needs to be done to understand the microbial mechanism behind the interaction between pH, substrate concentration and product distribution.

The production of SCOAs at high concentration is also expected to be beneficial to the subsequent separation and purification of the SCOAs. For some applications, such as bioplastics or microbial oil production the fermentation broth can be used without separation and purification [42, 43] . For other uses such as in the chemical, food, or pharmaceutical industry, separation and purification are required. Many processes have been proposed for separation and purification of SCOAs from aqueous mixtures such as fermentation broths,

e.g. membranes, electrodialysis, filtration, adsorption, distillation, electrocoagulation. [44, 45]. Some of the proposed recovery methods require an acidic pH in order to have most of the acids in their undissociated form. Moreover, higher product concentrations are desired to obtain higher recovery efficiency and reduce the separation costs. Therefore, working with highly concentrated substrates at uncontrolled acidic pH, as we have done in this study, has the potential to reduce the separation and purification costs. However, a full technical and economical analysis is required to assess the benefits of SCOA concentration and pH on the separation and purification process.

More in general, techno-economic analyses of the whole process, also including the separation and purification stages, are needed. Few reviews cover business cases [46, 47], providing an initial evaluation which however does not assess how each operating condition affects the economics of the downstream process. There is general agreement that further data and research are needed.

5 Conclusions

Anaerobic fermentation of highly concentrated model organic waste proved to be a promising strategy to produce SCOAs without pH control, by obtaining high product concentrations and avoiding the addition of chemical buffers. The increase of initial substrate concentration in the investigated range had a positive effect on the concentration and productivity of SCOAs and ethanol. All reactors showed similar yields, indicating no significant effect of the initial substrate concentration. Lactic acid was the main product in the four more concentrated substrate levels, followed by acetic acid in different proportions. In the reactor with the lowest initial substrate concentration, which had the highest pH, acetic acid was the main product, followed in equal measure by lactic, propionic, and butyric acids. No or little removal of the COD from the liquid phase was observed in all cases, which indicates very little, if any, production of methane or hydrogen. Removal of VSS and carbohydrates was higher for the more diluted feeds. The results indicate the need, in batch processes, of a compromise between productivity and yield, as shorter batch times gave higher productivities and longer batch times gave higher yields. In batch experiments with pH adjustment, ran with the most concentrated feed, higher pH values led to higher product concentration, yield and productivity without significant effect on the product composition. However, the cost of the chemical buffers should be taken into consideration and a cost-benefit analysis should be performed. Further studies should be carried out to optimise yields and productivities using continuous processes and to understand the interaction between substrate concentration and pH and their effect on product composition and process performance. Furthermore, in depth studies and analyses should be carried out to evaluate the economic feasibility of the proposed process.

Declarations

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Supervision: Davide Dionisi, Claudia Fernández Martín

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Table 1. Model substrate composition

Commercial name	Concentration (g l ⁻¹)
Organic wheatgrass powder	72.1
Yeast extract	80.0
Starch, soluble	45.7
Peptone, powder	26.0
D-Sucrose	66.6
Oleic acid, tech. 90 %	52.6

Table 2. Substrate characterisation

Substrate concentration	A	B	C	D	E
Dilution	1:1	1:2	1:4	1:8	1:16
pH	5.9	6.0	6.2	6.2	6.4
TCOD (g l ⁻¹)	394.6	197.3	98.7	49.3	24.7
SCOD (g l ⁻¹)	245.6	122.8	61.4	30.7	15.4
TC (g l ⁻¹)	172.1	86.1	43.0	21.5	10.8
SC (g l ⁻¹)	107.5	53.7	26.9	13.4	6.7
TSS (g l ⁻¹)	167.3	83.7	41.8	20.9	10.5
VSS (g l ⁻¹)	164.4	82.2	41.1	20.6	10.3
TS (g l ⁻¹)	321.1	160.5	80.3	40.1	20.1
VS (g l ⁻¹)	305.4	152.7	76.4	38.2	29.1
SCOD TCOD ⁻¹ (%)			62.2		
SC TC ⁻¹ (%)			62.4		

Table 3. Anaerobic sludge (inoculum) characterisation

Parameter	Value
pH	8.5
TCOD (g l ⁻¹)	43.4
SCOD (g l ⁻¹)	23.7
TC (g l ⁻¹)	18.9
SC (g l ⁻¹)	11.8
TSS (g l ⁻¹)	23.8
FSS (g l ⁻¹)	5.2
VSS (g l ⁻¹)	18.6
TS (g l ⁻¹)	26.7
FS (g l ⁻¹)	8.6
VS (g l ⁻¹)	18.1
SCOD TCOD ⁻¹ (%)	54.5
SC TC ⁻¹ (%)	62.4

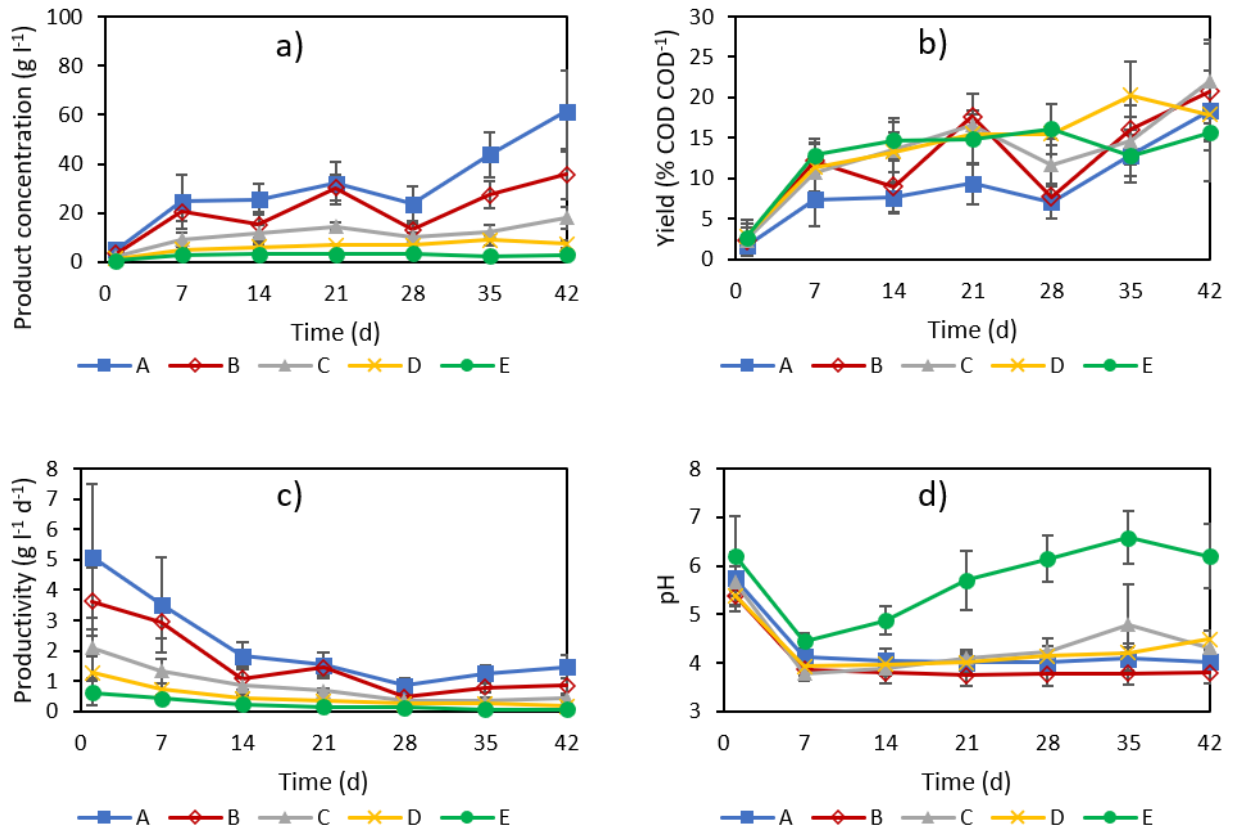


Fig. 1. Product concentration (a), yield (b), productivity (c) and pH (d) over time (average values with standard errors) of batch reactors A, B, C, D and E

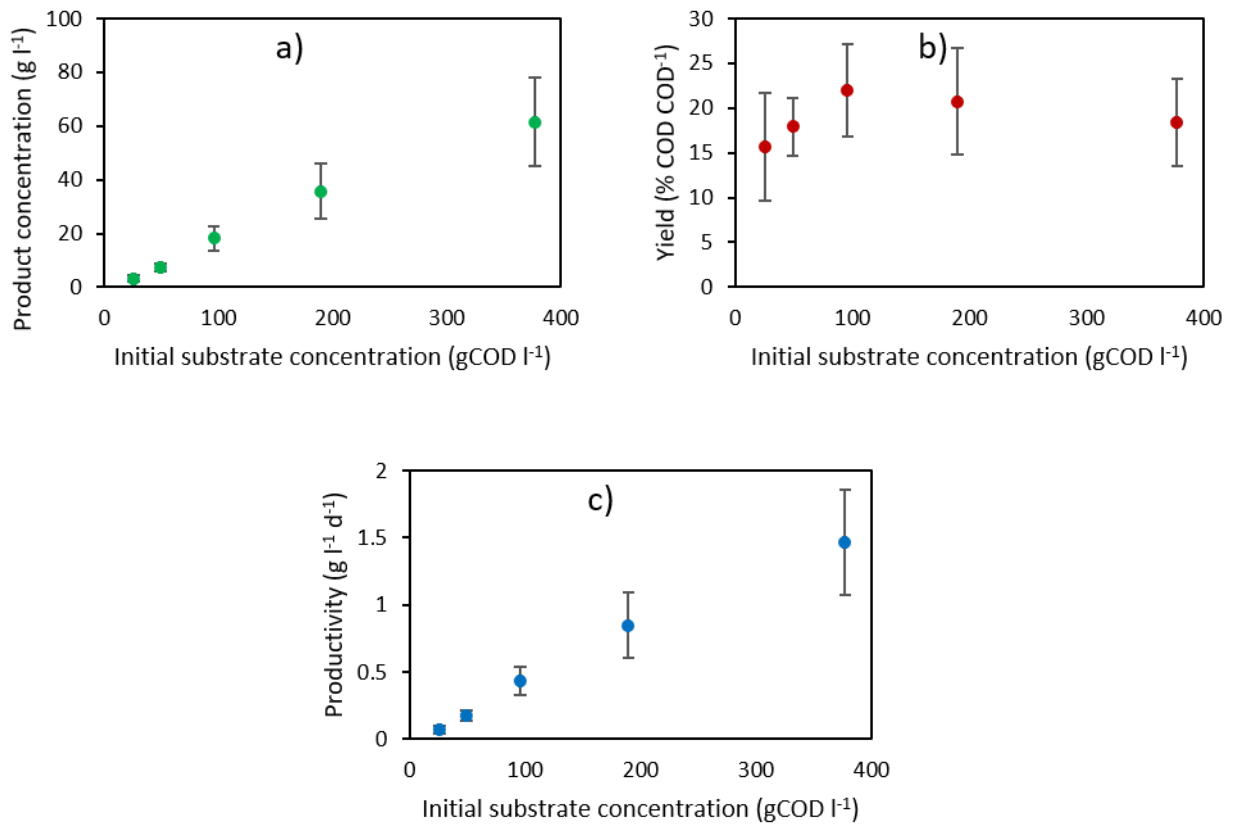


Fig. 2. Final (day 42) product concentration (a), yield (b) and productivity (c) (average values with standard errors) as a function of initial substrate concentration

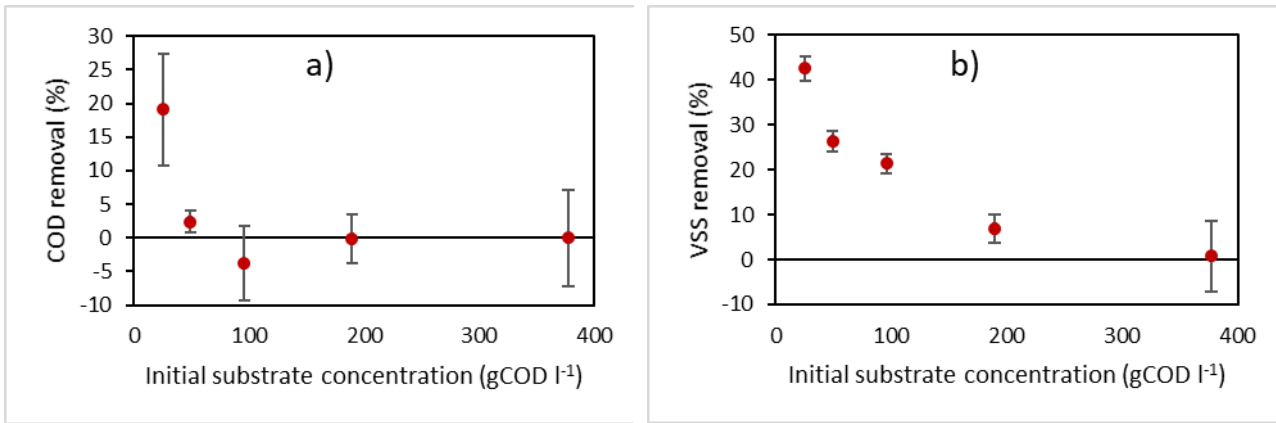


Fig. 3. Final (day 42) COD (a) and VSS (b) removal (average values with standard errors) as a function of initial substrate concentration

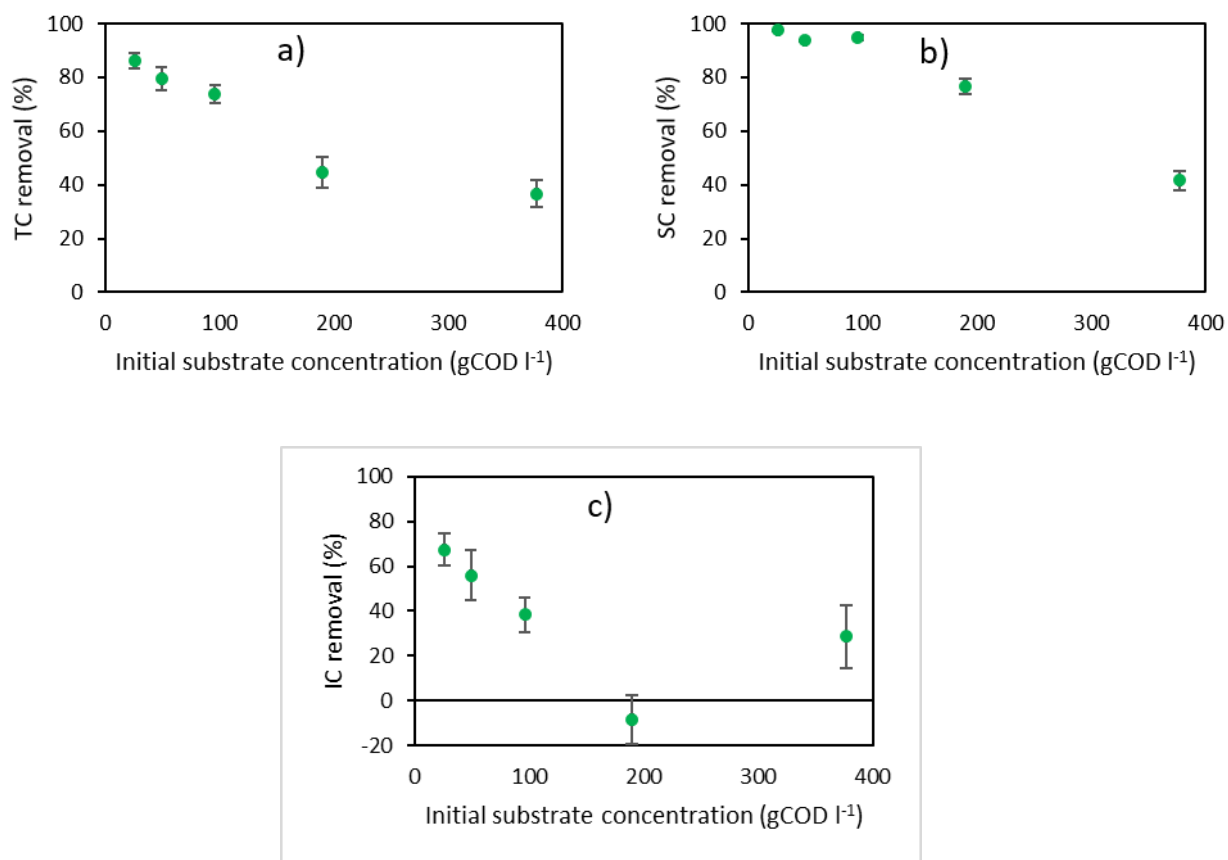


Fig. 4. Final (day 42) total (a), soluble (b) and insoluble (c) carbohydrates removal (average values with standard errors) as a function of initial substrate concentration

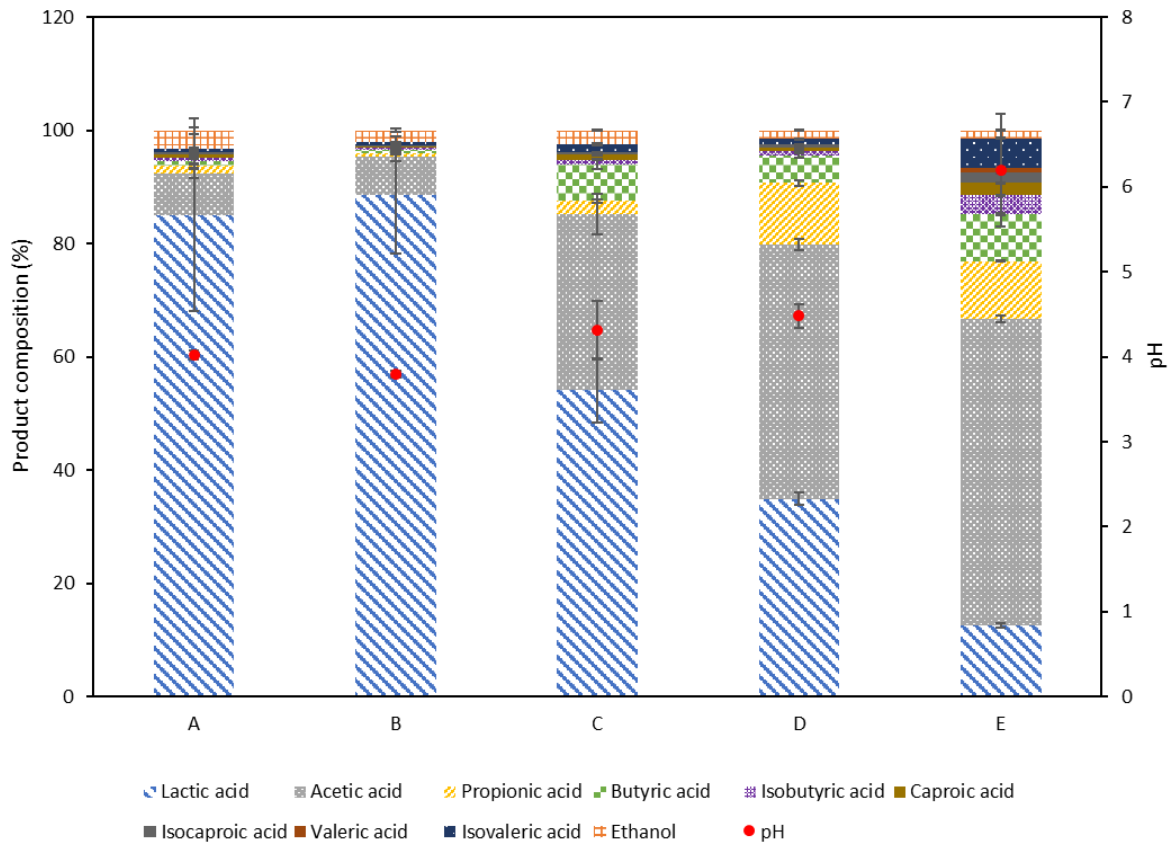


Fig 5. Final (day 42) product composition and pH (average values with standard errors) of batch reactors A, B, C, D and E

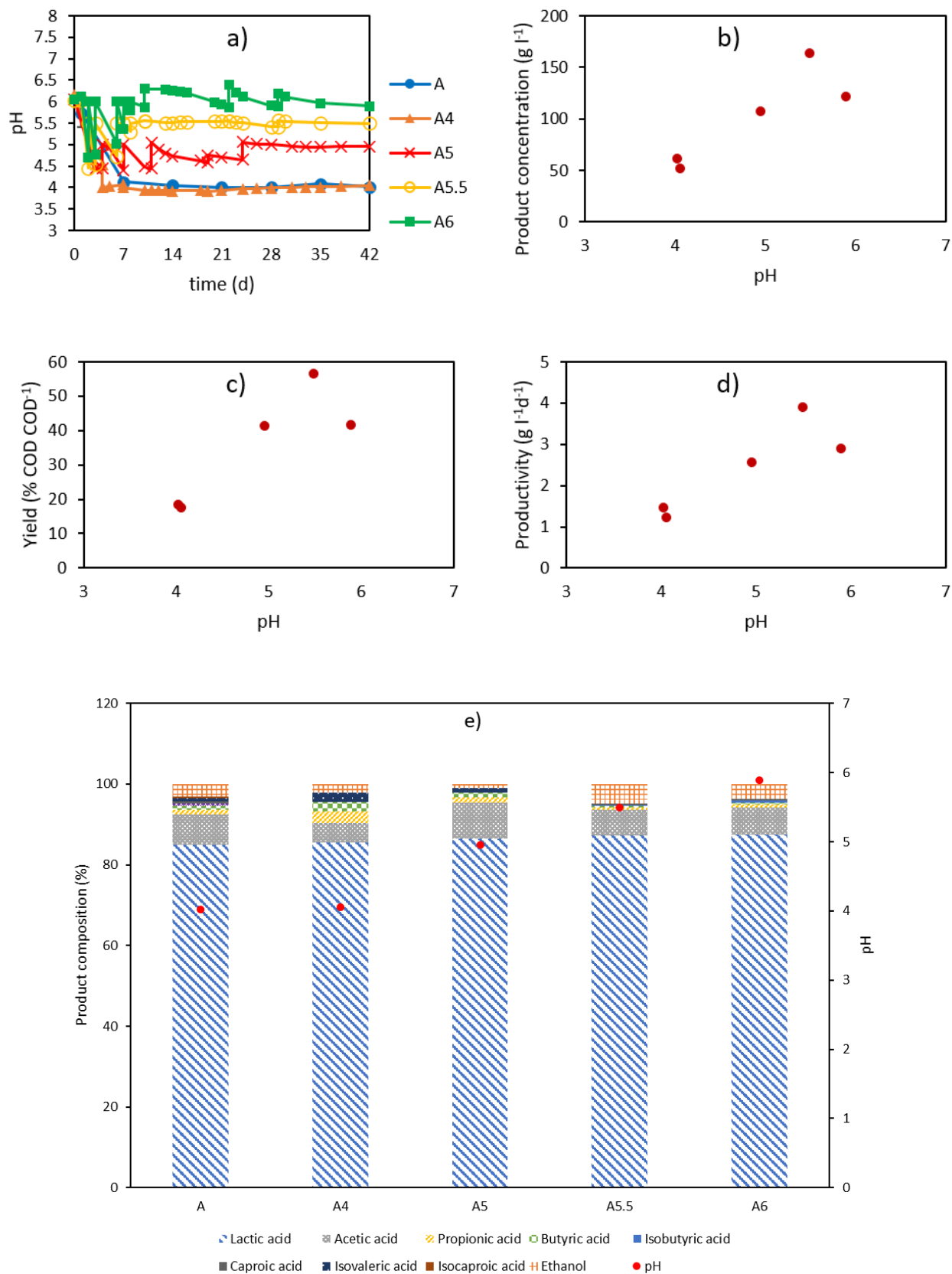


Fig 6. pH profile over time (a), total products (b), yield (c), productivity (d) vs pH at final day 42, and final product composition and pH (e) of batch reactors A (uncontrolled pH, average values), and of the reactors with adjusted pH A4, A5, A5.5 and A6.

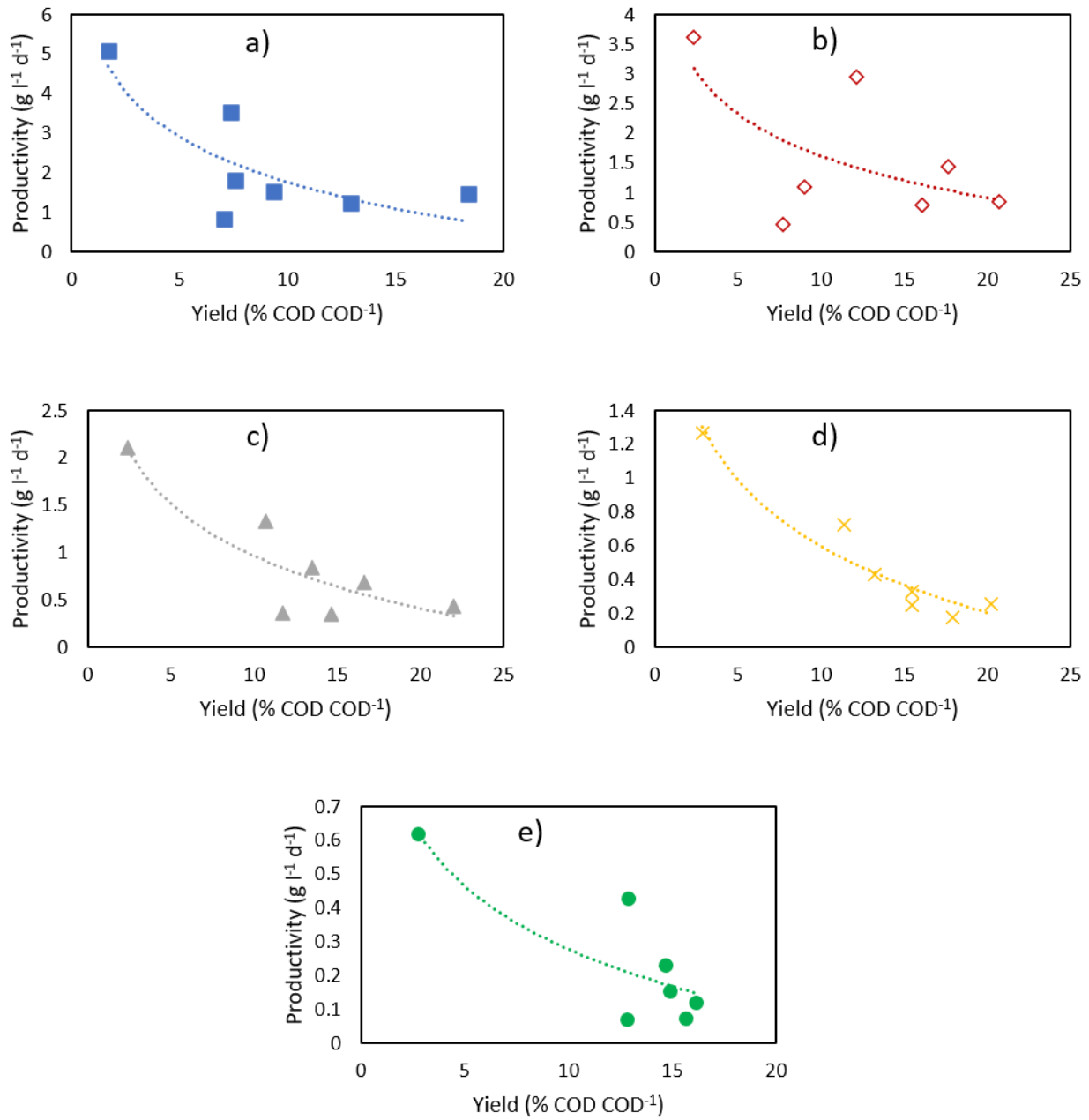


Fig 7. Productivity vs yield over time in batch reactors A (a), B (b), C (c), D (d) and E (e), using data from Figure 1. The trendline shows the general trend of the data.