OPTIMIZATION OF ACETATE PRODUCTION FROM CITRUS WASTEWATER FERMENTATION

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Abstract

Citrus wastewater is a sugar-rich waste stream suitable for the recovery of energy of material from its treatment. In this study, fermentation of citrus wastewater was carried out to assess the optimal conditions to maximize the bioconversion of the organic substrate into acetate. Unbalanced nutrient (C: N: P 200:0.1:0.1) enabled the highest acetate production. The presence of the particulate organic fraction enabled to obtain a higher acetate concentration regardless the initial COD concentration. Initial pH values higher than 5 did not cause substantial differences on the maximum bioconversion of COD into acetate in Trial 3, whereas pH lower than 5 hindered the hydrolysis process. Lastly, the bioconversion rate of the organic substrate into acetate decreased from a maximum of 23% to a minimum of 8% related to the initial COD. The achieved results demonstrated that the characteristics of citrus wastewater enable its valorisation without the need to apply energy-consuming processes.

1. Introduction

In the last decades, environmental legislations promoted resources recovery from waste and wastewater streams [1]. The will to apply the principles of the circular economy model in solid and liquid wastes management practices, has addressed researchers to employ innovative solutions aimed at enhancing waste streams valorisation, providing the simultaneous minimization of waste disposal and generation of value-added products [2].

The volatile fatty acids (VFA) are short-chain organic compounds constituted by a number of carbon atoms of six or fewer [3], which are produced during acidogenesis when organic matter is degraded under anaerobic conditions [4]. More precisely, three processes are involved in VFA production: hydrolysis, acidogenesis and acetogenesis. During hydrolysis, complex organic polymers are broken down into simpler organic monomers, which are subsequently fermented into acetic, propionic and butyric acids during acidogenesis [5]. Finally, during acetogenesis those products are converted into acetate.

VFA are valuable substrates that have a wide range of applications, like biogas production in anaerobic digestion process, in the food and pharmaceutical industries [3], carbon source for nitrogen and phosphorous removal in wastewater treatment plants or the synthesis of polyhydroxyalkanoates (PHA) for the production of bio-based plastics [6,7]. In all the above processes, the yield and the quality of the final products (i.e. biogas or PHA) are affected by the amount and composition of VFA deriving from the fermentation process [2,8]. Therefore, the maximization of VFA production is often critical for the performances of the downstream processes. According to the literature, during anaerobic fermentation many factors might affect VFA production [6,9]. Among these, environmental factors like pH and temperature, as well as operating parameter such as hydraulic retention time (HRT) and organic loading rate (OLR), have significant effects on the yield of VFA produced. The research findings suggest that the optimal values of the operating parameters for VFA maximization are highly dependent on the carbon source used. It seems that the proper operating parameters should be referred to the specific wastewater to be processed.

Besides the operating parameters, the production of VFA is substantially affected by the type of the waste/wastewater [12]. Indeed, researchers are still debating about which type of waste/wastewater is more suitable for VFA production. A suitable waste/wastewater should be characterized by high content of organic substrates (i.e. proteins, carbohydrates, fats, etc.), low nitrogen content, absence of toxic and recalcitrant compounds and high amount and availability of the waste itself [2,13].

Several solid and liquid wastes have been proposed in the literature for VFA production (i.e. paper mill effluents, cheese whey, kitchen waste, waste activated sludge, etc.) [2,11,14]. Among these, wastewater generated from agro-based production are potentially considered amongst the most suitable to maximize VFA production, because of the high content of organic matter (chemical oxygen demand – COD > 4000 mg/L) and the low content of nitrogen that could inhibit the fermentation process [15].

Citrus wastewater are organic-rich wastewater streams generated from citrus fruits processing, including washing of fruits and machineries, extraction of juice and essential oils, as well as peel drying and cooling [16]. Citrus wastewaters are characterized by high content of organic matter, suspended solids, essential oils and generally low pH (< 3), as well as unbalanced nutrients due to the lack in nitrogen and phosphorous [17]. The COD of citrus wastewater is generally high, ranging between 1000 mg/L and 10.000 mg/L depending on the type of citrus fruit processed [18]. Moreover, the COD is characterized by a very high biodegradability, being the readily biodegradable fraction close to 70% [19]. Based on the above characteristics, citrus wastewater is potentially suitable for VFA production. Most of the studies referred to citrus waste fermentation indicated that the presence of some potential toxic substances such as limonene and other essential oils present in the peels of citrus fruits could result in the inhibition of fermentation process [20,21]. Many of these substances are usually recovered before juice extraction since their great economic value and accordingly, their content in the wastewater deriving from juice processing is extremely low. For this reason, recovery of VFA from wastewater could be more convenient due to the lower amount of inhibiting substances.

However, as authors are aware, few studies dealing with VFA production from citrus wastewater, and in general aimed at its valorisation, have been reported in the technical literature so far [22,23].

In this light, the aim of the present study was to optimize VFA production from the fermentation of citrus wastewater. More precisely, this study investigated the role of some operating parameters like the nutrients (carbon to nitrogen to phosphorous – C: N: P) ratio, pH and the OLR on the VFA production from the fermentation of a citrus processing wastewater.

2. Materials and methods

2.1 Citrus wastewater characterization

Citrus wastewater samples were collected from a citrus processing industry located in Palermo (Agrumaria Corleone S.p.A.). Citrus wastewater was generated from tangerines processing and were characterized in terms of pH, electrical conductivity, total suspended solids (TSS) content, total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD) and VFA (as acetate) concentrations. The main features of the citrus wastewater are summarized in Table 1.

Table 1: Composition of the citrus wastewater

Parameter	Unit	Value
tCOD	[mg/L]	32,089
sCOD	[mg/L]	19,186
Total Nitrogen	[mg/L]	15.1
Total Phosphorous	[mg/L]	12.3
pH	[-]	3.21
VFA (as acetate)	[mg/L]	23.5
TSS	[mg/L]	1,247
Conductivity	[mS/cm]	1.47

2.2 Experimental set-up

Experiments were carried out in 1 L glass bottle reactors continuously mixed by a magnetic stirrer. The filling ratio with wastewater was close to 95% to reduce the headspace volume and thus limiting

the amount of oxygen that could dissolve in the liquid phase. Each reactor was subsequently hermetically sealed using a specific cap and a silicone gasket to avoid the air draw from the outside.

Four Trials were performed during the experimental campaign, each having a duration of 21 days. The reactors were fed in batch-static mode, meaning that neither feeding nor discharging (excepting the samples for analyses) were performed during the entire Trials duration.

In each Trial, only one operating parameter was changed, whereas the others were maintained constant. The operating parameter that provided the highest acetate production in the previous Trial was maintained constant during all the following Trials.

In Trial 1, the effect of carbon (as tCOD) to nitrogen and phosphorous ratio (C: N: P) was evaluated. To this aim, three different batch reactors were started-up with a different C: N: P ratio, equal to 200:0.1:0.1 (A1), 200:5:1 (A2) and 200:10:2 (A3). More precisely, in A1 no nitrogen neither phosphorous were added in the reactor, thus maintaining the original C: N: P ratio of the raw citrus wastewater, whereas in A2 and A3 ammonium chloride and potassium orthophosphate were added to obtain the desired C: N: P ratios. In each reactor, the raw citrus wastewater (no clarification) was diluted with tap water to obtain an initial tCOD concentration of 4000 mg/L and the pH was subsequently adjusted to neutral (7) by adding sodium hydroxide.

In Trial 2, the effect of the particulate organic matter was studied. In this case, the citrus wastewater was subjected to static settling for 2 hours and the supernatant was separated from the settled solids. The total suspended solids concentration in the supernatant was negligible (< 5 mg/L), thus it was considered that all the particulate substances were removed. Therefore, two reactors were started, the first one with the supernatant of the citrus wastewater subjected to static settling (2 hours) (B1), whereas the second one with the raw citrus wastewater, thus including the settleable solids content (B2). In both cases, the citrus wastewater, either clarified or not, was diluted with tap water to obtain the same tCOD concentration of the Trial 1 (4000 mg/L) and, similarly, pH was adjusted to neutral. Concerning the C: N: P ratio, it was set equal to A1 (no nitrogen and phosphorous supply) because it

resulted the best in terms of acetate production in the previous Trial, as better outlined in the following section 3.

In Trial 3, the effect of the initial pH was investigated. Based on the results obtained in the previous trials, the citrus wastewater (not clarified), with a C: N: P ratio equal to 200:0.1:0.1 and a tCOD of 4000 mg/L (after dilution) was added in four reactors (C1, C2, C3 and C4). Afterwards, the pH was adjusted to 7 in C1, 6 in C2, 5 in C3 and 4 in C4 by adding sodium hydroxide.

In Trial 4, the effect of the initial tCOD concentration was assessed. Four batch reactors were filled with not clarified citrus wastewater diluted with tap water according to different dilution factors, in order to obtain an initial tCOD concentration of 4000 mg/L (D1), 8000 mg/L (D2), 16000 mg/L (D3) and 32000 mg/L (D4). According to the best results achieved in Trial 3, the pH was adjusted to 7.

During Trial 1 the reactors were operated at room temperature (15 ± 2 °C), whereas in the other Trials, the reactors were maintained at controlled temperature (20 ± 0.1 °C). The pH was daily measured in all the Trials but was not controlled during the experiment excepting at day zero. A small volume (2 mL) was sampled daily from each reactor in all the Trials in order to measure the VFA (as acetate) concentration. No bacterial inoculum was added in the different trials since fermentative microorganisms were already present in the initial wastewater.

In Figure 1, a summary of the Trials operating conditions is reported.
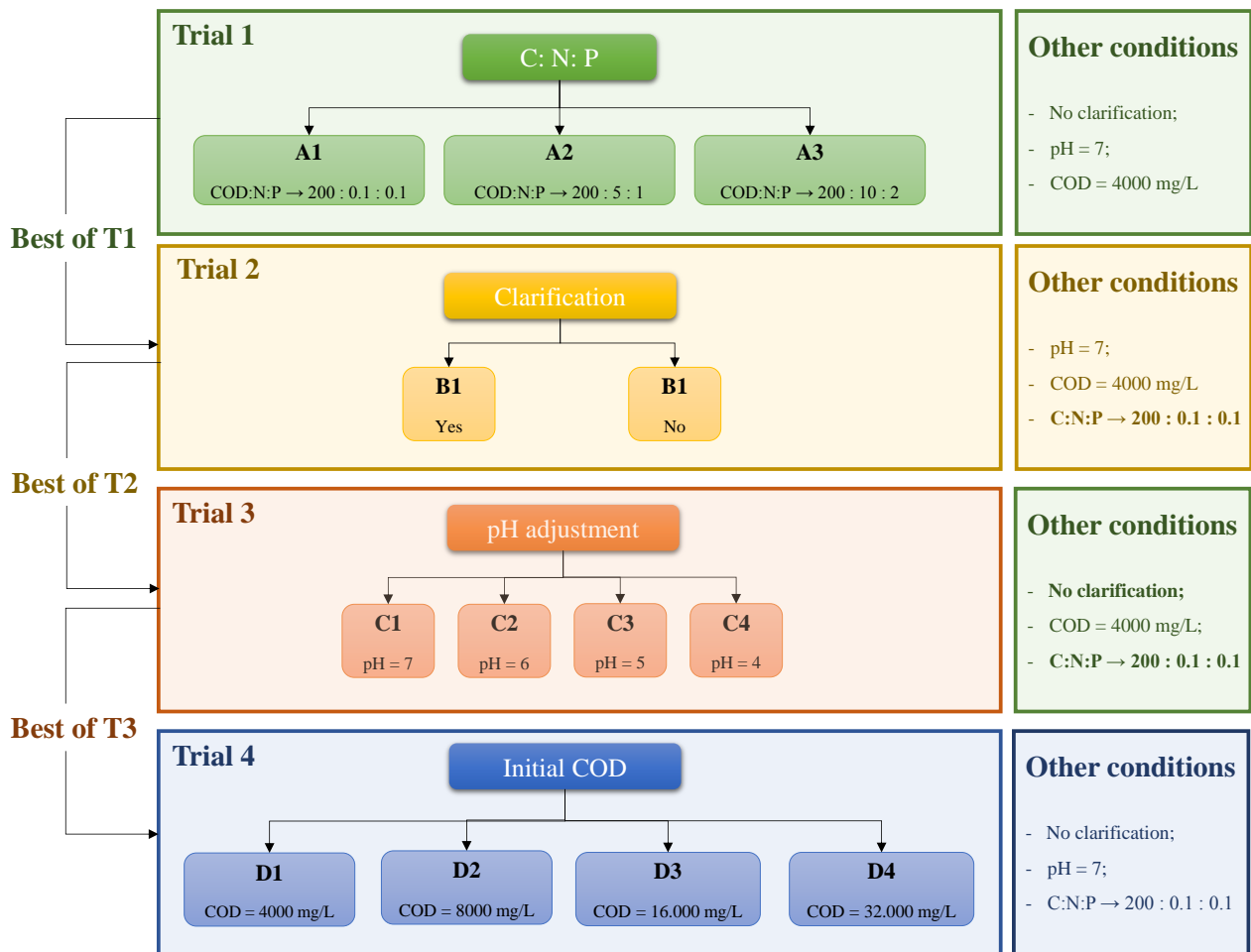
Figure 1: Summary of the operating conditions during the four Trials

2.3 Analytical methods

All the physical-chemical analyses, including TSS, sCOD, tCOD, total nitrogen (TN) and total phosphorous (TP), were performed according to Standard Methods [24]. The pH was measured using a pH-meter (WTW SenTix® 9xx) connected to a WTW 3430 multi-meter. NaOH was used in order to adjust the pH at the desired value. Samples from each reactor were filtered through a 0.45 µm membrane before measuring the acetate concentration by means of an ion-chromatograph (Dionex DX 120). A reference curve was constructed by using a pure standard of acetate in the range of 1-10.000 mg/L. For further details, the reader is referred to the literature [25].

3. Results and discussion

3.1 Effect of C: N: P ratio – Trial 1

In Trial 1, the effect of different C: N: P ratios on the acetate production was studied (Fig. 2). The initial acetate concentration in the citrus wastewater was about 20 mg/L. As depicted in Fig. 2a, the acetate concentration slightly decreased after the first day in all the reactors, but it significantly increased on day 2. More precisely, the acetate concentration raised from 1.90 mg/L to 601 mg/L, 576 mg/L and 520 mg/L in A1, A2 and A3, respectively. Thus, the maximum acetate concentration was achieved in the reactor operating under unbalanced nutrient condition (C: N: P = 200: 0.1: 0.1), although the difference in the acetate production was marginal (< 10-15%) compared to the other batch reactors. Similar results were found in the literature [26], in which the authors observed that nitrogen and phosphorus affected the acetate production in the acidogenic reactor. In particular, Boonsawang and co-authors [26] observed that the maximum production of acetate was obtained at COD:N:P of 100:1.1:0.5 treating biodiesel wastewater, whereas Fu and co-authors found that a C/N ratio of 30 maximized the acetate production from textile wastewater [27]. Similar results were also reported by Liu et al. [28], which observed that the maximum yield production of VFA was achieved under the highest C/N ratio investigated (50:1). Also, Huang and co-authors in a recent study observed that the yield of VFA production increased with C/N and the amount of acetate was proportional to the carbohydrate content of the waste subjected to fermentation [29].

At the same time, pH decreased in all the reactors from 7 to 5.4, 5.9 and 6.3 in A1, A2 and A3, respectively (Fig. 2b), indicating that the pH decrease was proportional to the acetate production. After day 2, the acetate concentrations decreased in all the reactors, although with different trends. Indeed, in A1 the acetate concentration decreased to a minimum value of 270 mg/L on the 7th day, whereas in A2 and A3 the acetate almost disappeared (< 15 mg/L). During this phase, the pH increased in all the reactors, reaching values of 6.80, 7.45 and 7.50 in A1, A2 and A3, respectively, when the acetate concentration was minimum. Atasoy et al. [6] reported that under no pH control, the acetate production is characterized by significant variation because of the development of different bacterial strains during the fermentation process. More recently, another study demonstrated that

under uncontrolled pH the acetate consumption could be observed because its production and degradation by methanogens could occur simultaneously [30]. However, operating at high C/N might minimize this effect, because it reduces the growth rate of methanogens [31,32].

After this transition period, the acetate concentration started to increase again in A1, although with a lower rate compared to the previous one. The maximum acetate concentration was reached on the 15th day (723 mg/L) and it remained almost stable at this value for about 5 days, after which it slightly decreased reaching a value of approximately 600 mg/L at the end of the observed period. In this phase, the acetate accounted for approximately 50% of the sCOD and 40% of the tCOD. Accordingly, pH decreased in this period, remaining stably close to 6 until the end of the trial. In contrast to what observed in A1, no further acetate production was observed in A2 and A3 after that the minimum value was reached and pH remained close to neutral in both the reactors.
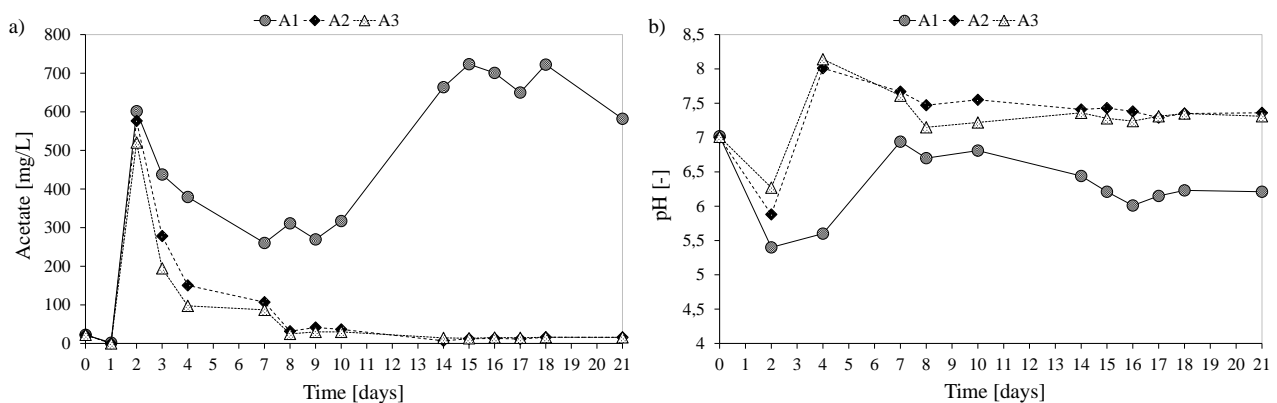
Figure 2: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater at different C:N:P in Trial 1.

The decrease of acetate concentration observed after the 2nd day was directly proportional to the C: N: P ratio. Indeed, in the literature it is widely reported as a condition of nutrients balance is favourable to the synthesis process of bacteria that might led to the consumption of the acetate produced during fermentation [33]. Therefore, in A2 and A3 the higher availability of nutrients likely favoured the growth of bacteria and, consequently, the higher consumption of the acetate previously

produced [30]. Nevertheless, even in A1 the acetate concentration decreased, thereby suggesting that the occurrence of bacterial growth phenomena, even if minimal.

It is worth to observe that after the 2nd day, the pH increased in all the reactors, suggesting the occurrence of hydrolysis reactions involving the particulate organic fraction in the citrus wastewater, according to literature [34]. Indeed, the recovery of the acetate production in A1 was due to the fermentation of the hydrolysed products previously produced. Because the hydrolysis process involves complex reactions, the production rate of the soluble organic molecules ready to be fermented is low [2]. This certainly limited the rate of acetate production that was lower compared with that observed until day 2, when the organic matter available for the fermentation process was already in the soluble form. In A2 and A3, the organic matter deriving from the hydrolysis of the particulate one was likely simultaneously used by bacteria for growth purpose, thus reducing its availability for the fermentation reactions. In A1, the consumption of the acetate by bacteria was minimized because of the low C: N: P, thereby favouring the fermentation reactions over new biomass synthesis.

Based on the above results, unbalanced nutrient condition is preferable for the acetate production from citrus wastewater fermentation. Indeed, it allowed achieving a higher bioconversion of both the soluble and particulate COD into acetate, while preventing the use of the organic matter by bacteria for growth. The above results are consistent with the current literature results, highlighting that unbalanced nutrient condition (high C/N) are favourable not only to promote the VFAs production but also to suppress the consumption of VFAs [6,29,31].

3.2 Effect of settleable solids removal – Trial 2

In Trial 2, two batch reactors with raw (B1) and clarified (B2) citrus wastewater were started. Figure 3 shows the trend profiles of the acetate concentration (Fig. 3a) and pH (Fig. 3b) during the experiment.
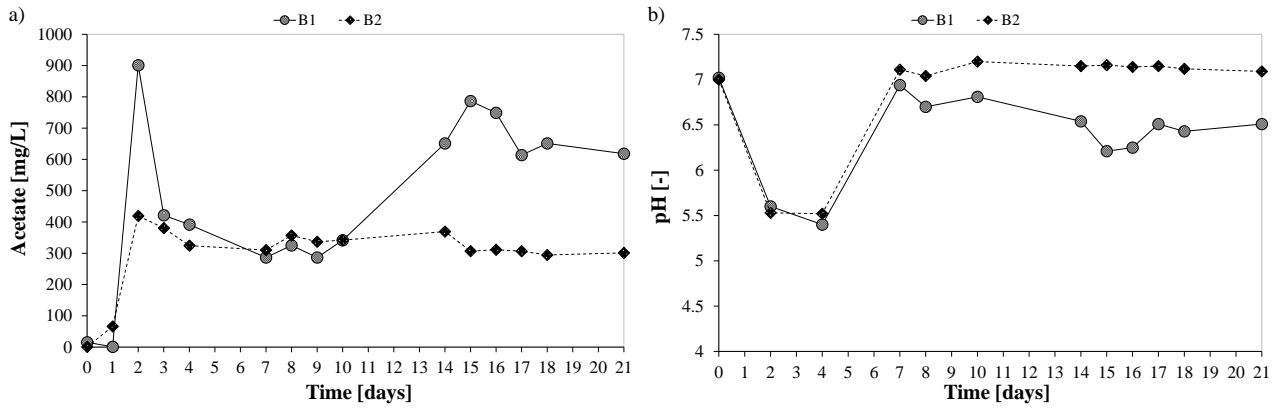
Figure 3: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater with and without suspended solids in Trial 2.

The trend of the acetate concentrations and pH in B1 were very similar with those observed in A1 because the operating conditions were basically the same. This confirmed the repeatability and reproducibility of the achieved results. In more detail, as reported in Fig. 3a, a first peak in the acetate concentration was observed on the 2nd day of operation, according with that observed in Trial 1. However, in this case the concentration of the acetate was approximately 50% higher (900 mg/L vs 600 mg/L), likely due to the higher temperature in B1 (20 °C *vs* 16 °C). Hereafter, the decrease of the acetate concentration and its further increase due to hydrolysis of the particulate substrate nearly followed that observed in A1. The trend of the acetate concentration in B2 rapidly increased at the beginning of the experiment, reaching the maximum value of 419 mg/L on day 2. In the three following days, the acetate concentration slightly decreased by approximately 13% (357 mg/L on the 5th day), while remaining almost constant until the end of the observed period. Although the initial tCOD concentration was the same in B1 and B2, the highest value of acetate concentration was observed in B1. The reason for this result could be twofold. First, in B2 bacteria were present in a lower amount in the reactor because, being mainly in particulate form, were removed during the sample pre-treatment, thus reducing the efficiency of the acidification process. In this way, the solids removal caused a decrease of the indigenous biomass responsible of fermentation and, as a result, the reduction of acetate production. This results is consistent with recent studies, in which the authors

reported that a no acclimated inoculum could led to a lower yield of VFA production [32,35]. Second, it could be possible that the hydrolysis of the particulate organic matter occurred also in the early stage of the experiment in B1, leading to a higher availability of soluble products to be fermented [36]. In this light, literature results reported that low availability of acetate gives a competitive advantage to methanogens bacteria given their lower growth rate compared to that of acidifying bacteria [31]. Therefore, if the acidogenesis process is slow, simultaneous acetate consumption by methanogens could occur.

Moreover, it is worth noting that the decrease of the acetate concentration in the long-term was lower in B2 (> 65% in B1 vs 13% in B2), thereby indicating that the removal of the particulate matter could beneficial in preventing the acetate deterioration. This result confirmed that inhibition of methanogens bacteria, or their removal as in this case, could be an effective strategy to suppress the acetate consumption [31].

The results obtained in Trial 2 highlighted the role of the particulate organic matter in acetate production by the fermentation process. Indeed, on the one hand the presence of the particulate organic fraction enabled to obtain a higher acetate concentration independently of the initial COD concentration, but on the other hand, it was likely responsible for the acetate decrease in the long-term.

Based on the above results, a possible operating strategy could be the separation of the particulate matter after the achievement of the first peak of the acetate concentration. Although a similar result in terms of the acetate production yield was achievable after approximately 15 days, this strategy would require a higher storage capacity for the fermenting reactors, hence higher investment costs.

3.3 Effect of the initial pH – Trial 3

In Trial 3, the effect of the initial pH value was evaluated. Figure 4 depicts the trends of the acetate concentration (Fig. 4a) and pH (Fig. 4b) during the Trial 3.
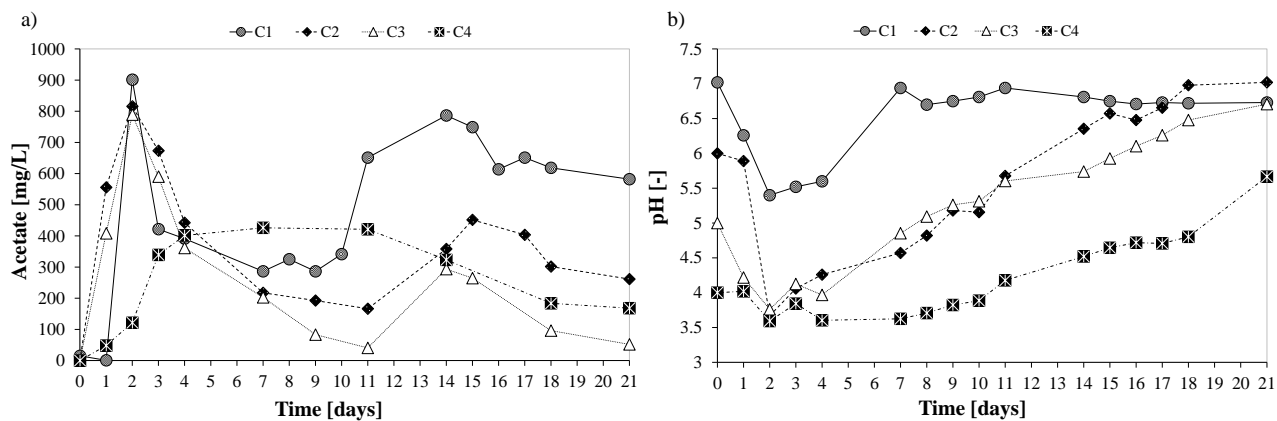
Figure 4: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater at different initial, pH in Trial 3.

In this trial, the reactor C1 was not started because the operating conditions were the same of B1 and reproducibility of the test was previously demonstrated. Therefore, the data achieved in the previous trial referred to the reactor B1 were considered as the same of C1 in Trial 3. A similar behaviour was observed in C1, C2 and C3 in terms of acetate production during the entire experiment. More precisely, the first peak was noted after 2 days in all reactors. The maximum acetate concentration was very similar in the above cited reactors (800-900 mg/L), although showing a slighter decrease with lower pH values, confirming that low pH likely hindered fermentation reactions [37]. In contrast, the minimum value of pH, measured on day 2, was different in C1, C2 and C3 (Fig. 4b). Indeed, the lowest pH values were measured in C3 (3.68) and C2 (3.80), whereas it was higher in C1 (5.4). Hereafter, pH increased in all the reactors, although in C2 and C3 it was constantly lower than C1. This result likely affected the hydrolysis processes in C2 and C3 and, consequently, the recovery of the acetate production after the 10th day was lower compared to C1. In particular, the maximum concentrations achieved in C2 and C3 were approximately 50% (410 mg/L *vs* 790 mg/L) and 70% (260 mg/L *vs* 790 mg/L) lower compared to that of C1. This result indicated that low pH are not favourable to the hydrolysis process, thereby confirming what reported in the literature [27,38]. In contrast, the trend of acetate production in C4 was completely different with those above discussed. Indeed, the acetate concentration increased very slowly at the beginning of the experiment, reaching

a maximum value close to 400 mg/L on day 4. Subsequently, this concentration remained almost constant for about 7 days, while showing a slow decrease afterwards, almost halving at the end of the experiment. In contrast with the other reactors, the recovery of the acetate production due to hydrolysis of the particulate organic matter was not observed in C4. This result confirmed that low pH values are not favourable for the hydrolysis process and, even, at pH lower than 4, this was completely inhibited [37,38]. Another difference was observed in terms of acetate decrease after the first peak. Indeed, while in C1, C2 and C3 the acetate concentration decreased proportionally to the pH decrease, in reactor C4, instead, the decrease of the acetate concentration was smaller in terms of absolute value compared with the other reactors ($< 45\%$) and it showed a slow decrease in time according to what previously discussed.

The aforementioned results are in good agreement with previous literature results, revealing that neutral or alkaline conditions can potentially accelerate the solubilization of organic particles, whereas acidic conditions can inhibit the process [39].

Based on the above results, it can be stated that the maximum production of acetate on the soluble COD (first peak) was comparable in C1, C2 and C3, whereas in C4 it was significantly lower. These results suggested that initial pH values higher than 5 did not cause substantial differences referring to the maximum bioconversion of COD into acetate, whereas pH lower than 5 caused a slowdown of the start-up of fermentation reactions. Moreover, the acetate deterioration increased with the pH decrease in the range of 5-7, whereas at lower pH it was shifted in time and was lower in percentage terms. Lastly, the hydrolysis process was hindered with the pH decrease, while it completely stopped at pH lower than 5. As reported in the literature, pH of 7 was found the most suitable for hydrolysis and acidogenesis of kitchen waste, leading to the highest VFAs concentrations in comparison with other pH values [40].

The above results are consistent with previous literature, in which it was demonstrated that under uncontrolled pH conditions the acetate production is variable during the fermentation process and the highest acetate production was achieved at pH close to 7 [30]. Moreover, these results also agreed

with the previous finding that the neutral conditions could improve the hydrolysis efficiency of organic substrates [41].

3.4 Effect of the initial COD concentration – Trial 4

The effect of different initial COD concentrations was evaluated in Trial 4. Figure 5 depicts the trend profiles of the acetate concentration (Fig. 5a) and pH (Fig. 5b) as well as the relationships between the COD with the maximum acetate production and the maximum acetate bioconversion rate (Fig. 5c).
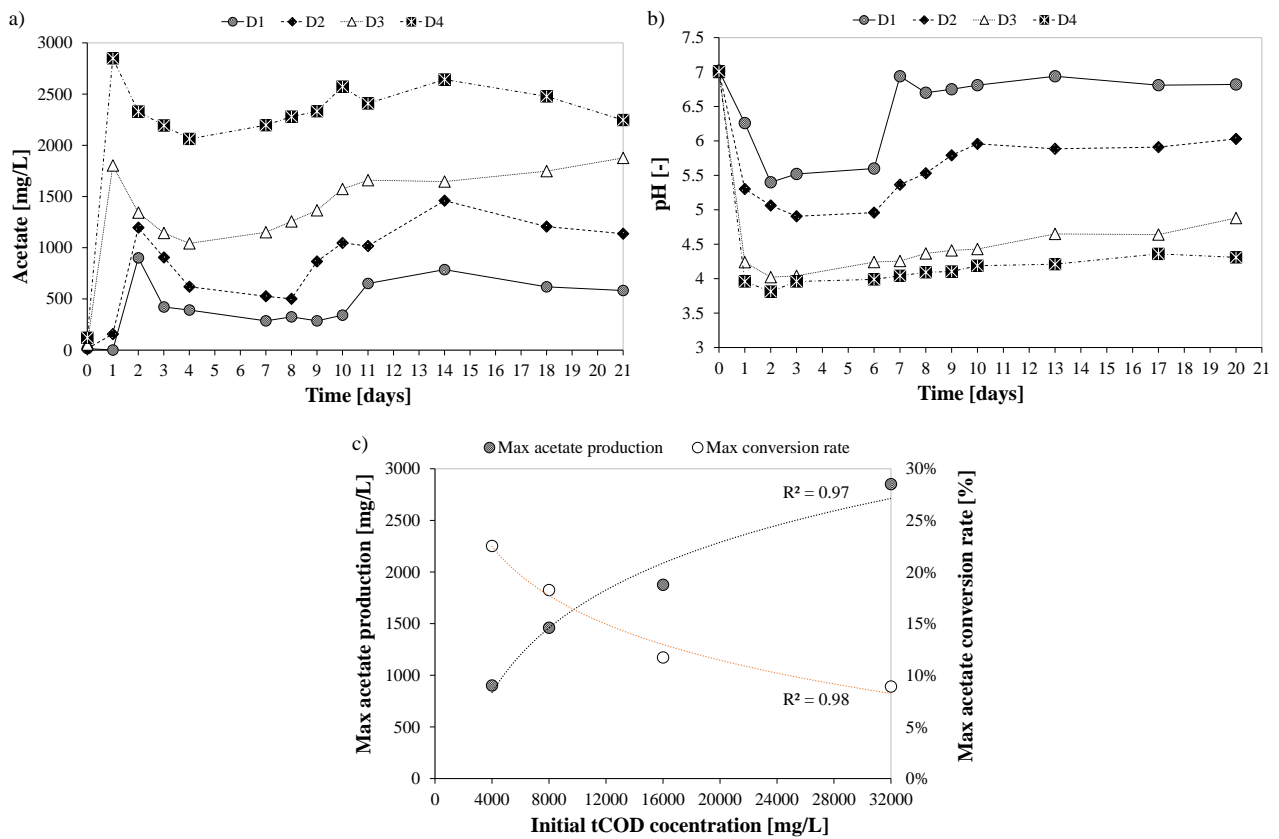
Figure 5: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater at different initial tCOD concentration in Trial 4; maximum acetate concentration and maximum bioconversion rate in relationship with the initial tCOD (c).

For the same reason explained in Trial 3, the data referred to the reactor D1 are the same of B1 (Trial 2). As reported in Fig. 5a, the trends of the acetate production were similar in D1, D2 and D3, whereas

it was slightly different in D4 especially in the second phase of the experiment. More in detail, as observed in the previous trials, a first peak in the acetate production from the sCOD was observed in the very first days after the start-up of the experiment. In particular, the maximum acetate production was observed in D4 (2850 mg/L), followed by D3 (1403 mg/L), D2 (1195 mg/L) and, lastly, D1 (901.2 mg/L), thereby highlighting a certain relationship with the initial tCOD concentration. Moreover, it was noted that the maximum acetate production occurred earlier with the increase of the initial tCOD concentration. Indeed, in D3 and D4, the maximum acetate concentration was observed after one day from the beginning of the experiment, whereas in D1 and D2 after two days. Hereafter, as also observed during the previous trials, in all the reactors the acetate concentration slightly decreased. Referring to the pH trend (Fig. 5b), the drop of pH occurring during the early stage of the experiment was proportional to the initial COD concentration. Therefore, the minimum pH values were observed in D4 (3.81) and D3 (4.01), whereas in D2 and D1 it was higher and close to 5 and 5.4 in D2 and D1, respectively. According to what observed in Trial 3, the decrease of the acetate concentration was smaller at low pH. Therefore, it could be stated that the smaller rates of the acetate decrease observed in D4 and D3 were due to the lower pH values. Hereafter, the acetate concentration increased in all the reactors due to hydrolysis of particulate COD. In this case, the acetate production was not proportional to the initial tCOD. Indeed, compared to the minimum value in each reactor, the acetate concentration increased by approximately 20% in D4, 45% in D3, and about 200% in D2 and D1. Even in this case, the reason of this result could be due to the lower pH values in D3 (close to 4) and D4 that somehow hindered the hydrolysis processes.

The maximum acetate concentration obtained in each reactor was also related to the initial tCOD concentration. As shown in Fig. 5c, the maximum acetate production did not increase proportionally to the initial tCOD concentration and the relationship was asymptotic. Similarly, the maximum bioconversion rate of the tCOD to acetate decreased with the initial COD concentration.

This meant that the increase of the initial COD concentration is not associated to a higher bioconversion rate of the organic substrate to acetate. The above results were consistent with previous

literature and in particular with the results reported by Kaushalya et al. [42]. In more detail, the authors referred that an increase in VFA production was observed as the organic loading rate increased, although the increment was not proportional at each step. In another study, the authors observed that the acetate production increased with OLR although the conversion yield of COD to acetate decreased proportionally with the OLR [43]. According to the authors findings, the higher is the COD concentration the higher is the reduction in the kinetic of VFA production. Consequently, proper COD concentration should be carefully ensured to enable maximization of acetate production.

As previously discussed, this result is likely due to an excessive decrease of the pH observed in those reactors with the higher initial tCOD concentration. It is reasonable to state that the limiting process was the hydrolysis of the particulate COD, due to the low pH, that likely reduced the enzymatic ability to hydrolyse the organic substrate. Indeed, Cheah and co-authors [34] demonstrated that at high COD concentration, VFA production was not inhibited if pH was controlled at approximately 6. Therefore, in this study, the simultaneous high COD concentration and the uncontrolled pH during fermentation led to the gradual decrease in VFA production because of acidogenic bacteria inhibition due to pH.

In this respect, a control of the pH during the fermentation process could be beneficial to foster the establishment of the proper conditions for hydrolysis. The above results are consistent with the literature. Indeed, VFA concentration produced from different types of wastewater rarely increased with the initial COD concentration, whilst it was found that the maximum of VFA production was achievable only within a specific range dependent on the wastewater and other operating conditions [2,6].

3.5 General considerations for practical implementations

The results obtained in the above discussed trials, indicated that environmental and operating conditions could significantly affect the acetate production from the fermentation of citrus wastewater.

The unbalance of nutrients, typical for citrus wastewaters, is beneficial for maximizing the acetate production. Certainly, the unbalance of nutrients could cause severe issues for the treatment of such wastewater, thus the dosage of nutrients (nitrogen and phosphorous) should be performed only after the fermentation process.

Regarding the pre-treatment through clarification, it was observed that this would reduce the maximum acetate production, but at the same time, it might prevent the acetate decrease in the long-term. Moreover, the removal of the particulate matter hindered the chance to recover acetate from the fermentation of the particulate organic fraction. Therefore, a possible and advisable operating strategy could be the separation of the soluble and particulate fractions after some operating days, as suggested by Conca et al. [44]. In this way, the soluble fraction could be immediately used or stored without any risks of acetate deterioration. In contrast, the particulate fraction could be left to ferment, although this would imply a higher volume of the fermenter reactor. At the end of this phase, the fermented wastewater should be filtered to separate the citrus wastewater enriched in acetate from the residual particulate fraction.

Regarding the pH, it was observed that the maximum acetate production on the soluble fraction of the COD was independent of pH in a range of 5-7. Based on the achieved results, it would be convenient to carry out the first stage of the fermentation at low pH (5), because the raw citrus wastewaters have pH close to 3 and this would require a low amount of buffer reagents to adjust the pH. However, to favour the hydrolysis of the particulate fraction, pH should be later adjusted to neutral values.

The initial tCOD concentration affected the maximum acetate production, although the bioconversion rate significantly decreased at high COD. However, from an operating point of view, the management of the initial tCOD concentration is not easy to perform, because it depends on the production processes and on the kind of fruits that change periodically and unpredictable. The adjustment of the initial tCOD concentration is also a crucial step prior to perform the biological treatment of citrus wastewater, in order to avoid an overload of the biological system. Practically, this is commonly

obtained by mixing different streams having different COD values, but obviously, this would imply a high storage capacity within the production site. The same practise could be implemented with the aim to maximize the COD bioconversion to acetate. Referring to the acetate production after hydrolysis, it was observed that the lower production achieved in the reactors operating with higher COD was likely due to the low pH. Indeed, as observed in Trial 3, at pH lower than 4, the hydrolysis of the particulate matter was limited. Thus, the pH adjustment could prevent the slowdown or shutdown of the hydrolysis process, thereby making available also the acetate fraction achievable from the particulate COD.

Based on what discussed so far, a possible process scheme could involve the following steps:

- adjustment of the initial COD concentration to a value close to 4000-5000 mg/L;

- initial pH adjustment to 5 and neither clarification or nutrients dosage (nitrogen and/or phosphorous);

- solid/liquid separation after approximately 48 hours (with the liquid fraction ready to be used or stored);

- dosage of alkaline reagents (pH to 7) in the fermenter reactor with the particulate matter.

A conceptual scheme based on the above considerations is reported in Figure 6.
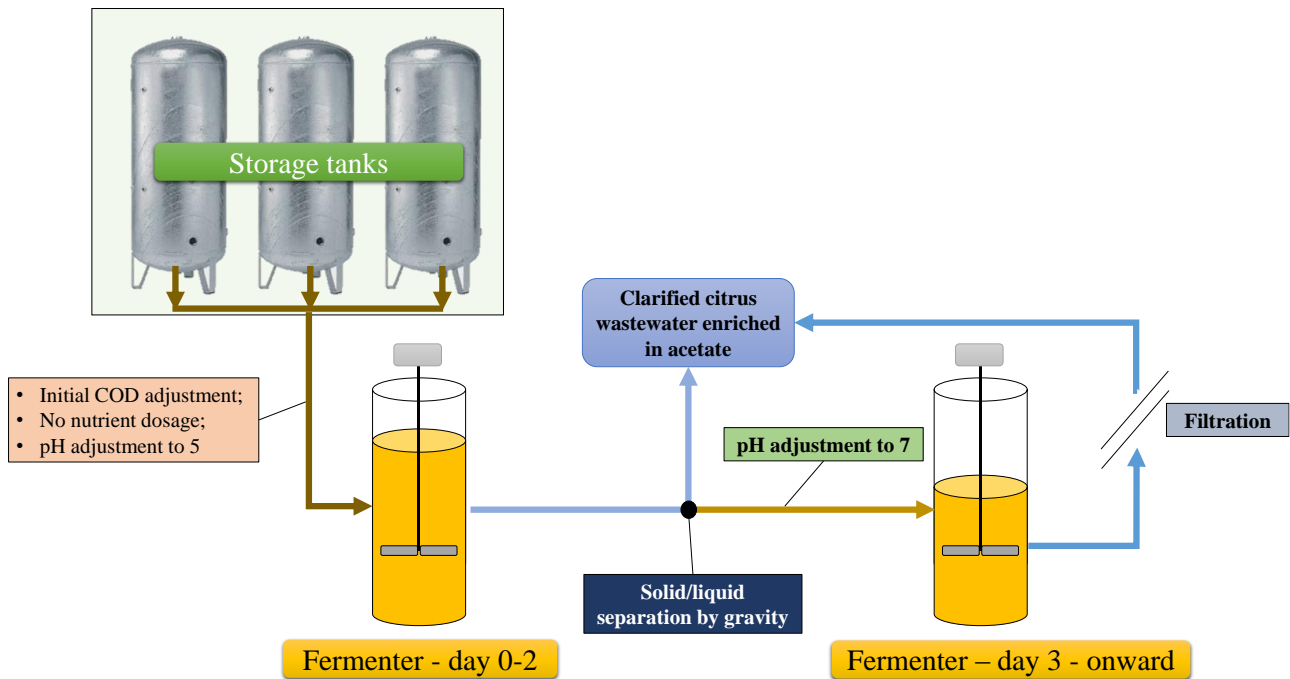
Figure 6: Conceptual scheme for maximizing of the acetate production from citrus wastewater

Conclusions

In this study, the effects of some operating parameters on the production of acetate from citrus wastewater were evaluated. The production of acetate was significantly affected by all the investigated parameters. In particular, the nutrients unbalance and the presence of the particulate fraction were found to be beneficial for maximizing the acetate production. Moreover, initial low pH (close to 5) are preferable to obtain high acetate production in the short-term, while avoiding excessive operating costs for pH adjustment. Furthermore, pH close to neutral should be ensured in the long term to enable the hydrolysis of the particulate organic fraction. The clarification of the citrus wastewater prior to fermentation prevented the decrease of the acetate concentration in the long-term, although reducing the maximum acetate production. Lastly, the bioconversion rate from COD to acetate significantly decreased at high COD, thus suggesting the advisability to operate at low initial tCOD concentration (< 5000 mg/L). It should be stress that many of the above conditions are typical for citrus wastewaters. This enables the valorisation of these waste streams, without the necessity of applying expensive and energy-consuming processes.

Acknowledgments

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Figure caption

Figure 1: Summary of the operating conditions during the four trials

Figure 2: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater at different C:N:P in Trial 1.Figure 3: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater with and without suspended solids in Trial 2.

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Figure 4: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater at different initial pH in Trial 1.

Figure 5: Acetate concentration (a) and pH (b) in fermentation of citrus wastewater at different initial tCOD concentration in Trial 4; maximum acetate concentration and maximum bioconversion rate in relationship with the initial tCOD (c).

Figure 6: Conceptual scheme for maximizing of the acetate production from citrus wastewater

Table caption

Table 1: Composition of the citrus wastewater