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Research article

Co-production of ethanol and biodiesel from sweet sorghum juice in two consecutive fermentation steps

Carlos Rolz *, Roberto de León, Ana Luisa Mendizábal de Montenegro

Biochemical Engineering Center, Research Institute, Universidad del Valle de Guatemala, Guatemala

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ABSTRACT

Background: Sugars from sweet sorghum stalks can be used to produce ethanol and also to grow oleaginous yeasts. Instead of two separate processes, in this paper we propose a different route producing ethanol and microbial oil in two consecutive fermentation steps.
Results: Three yeasts were compared in the first ethanol producing step. In the second step four different oleaginous yeasts were tested. Sweet sorghum juice was first clarified and concentrated. High gravity ethanol fermentation was carried out with concentrated juice with 23.7 g/100 mL of total sugars and without added nutrients. Total sugars were 2.5 times more than the original clarified juice. One yeast gave the best overall response over the two other tested; relative high ethanol productivity, 1.44 g ethanol/L·h^{-1}, and 90% of sugar consumption. Aeration by flask agitation produced superior results than static flasks for all yeasts. Microbial oil production was done employing the residual liquid left after ethanol separation. The pooled residual liquid from the ethanol distillation contained 7.08 g/mL of total carbohydrates, rich in reducing sugars. *Trichosporon oleaginosus* and *Lipomyces starkeyi* produced higher dry biomass, total sugar consumption and oil productivity than the other two oleaginous yeasts tested; with values around 25 g/L, 80%, and 0.55 g oil/L·h^{-1} respectively. However, the biomass oil content in all yeasts was relatively low in the range of 14 to 16%.
Conclusion: The two step process is viable and could be considered an integral part of a consolidated biorefinery from sweet sorghum.
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1. Introduction

Sweet sorghum is a sugar rich crop that has been considered an alternative feedstock to sugarcane for first generation ethanol production due to its efficient C4-photosynthesis, short production cycle, and nitrogen and water use efficiency, high tolerance to environmental stress and adaptability to marginal lands [1]. Sweet sorghum presents additional advantages: (a) it can be considered a multiproduct crop due to its high sugar productivity and its grain with adequate nutritional characteristics [2], (b) it can sustain a full year production cycle as sweet sorghum is capable of multiple ratoon crops [3], and (c) it can be grown with compost addition and a minimum amount of chemical fertilizers [4] or the addition of organic soil amendments [5]. However, in recent work it has been reported that the harvested stalk sugar content deteriorates rapidly at ambient temperatures and the proportions of individual sugars change with negative consequences for further processing [6,7]. As a consequence, and due to the short harvest window, a practical suggestion to extract, clarify, and concentrate the juice and store the syrup for subsequent fermentation has been proposed [8].

Sweet sorghum has been studied extensively as a raw material for ethanol production [9]. Moreover, several authors have proposed the use for ethanol production not only of the stalk soluble sugars but also of those carbohydrates produced by the hydrolysis of the bagasse and leaves [10,11]. On the other hand, several researchers have investigated the very high gravity (VHG) ethanol fermentation from sweet sorghum syrups [12,13] in order to find the maximum sugar conversion into ethanol and the minimization of fermentation byproducts, by testing different ethanol tolerant yeasts, several nitrogen sources and adding other nutrients. VHG ethanol fermentation fits well within the upstream concept of sweet sorghum juice concentration and storage.

A general interest in producing microbial oils for further transformation into biodiesel has identified promising oleaginous algae, bacteria, fungi and yeast and has encouraged studies concerning their

* Corresponding author.
E-mail addresses: carlosrolz@uvg.edu.gt (C. Rolz), ldeleon@uvg.edu.gt (R. de León), mendiza@uvg.edu.gt (A.L.M. de Montenegro).

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growth and lipid accumulation kinetics [14,15,16,17]. Sweet sorghum juice, syrup and hydrolyzed bagasse have been used as carbon substrates for microbial oil production mainly by yeasts [18,19,20]. Oleaginous yeasts have been also grown on sucrose, glucose and fructose containing raw materials, specially sugarcane and beet molasses [21,22,23]. In all cases the resulting microbial biomass grown in optimal conditions had high lipid content.

Instead of employing sweet sorghum juice as raw material utilizing different processes in order to produce, either ethanol or microbial oil, as was described above, a different alternative route is possible. The strategy employs VHG fermentation so that an acceptable ethanol concentration is reached, and also, in order to leave enough residual sugars for the subsequent oleaginous yeast growth. In other words, a consolidated sweet sorghum juice process scheme is generated in which two consecutive fermentation steps take place: ethanol in the first one and microbial biomass enriched with oil in the latter step [24]. The process strategy is illustrated as a block diagram in Fig. 1.

The objective of the present work was directed to procure insight to the following matters: a) what will be the individual sugar distribution after the ethanol fermentation by different yeasts of clarified and concentrated sweet sorghum juice under VHG conditions with no nutrients added? and b) how will various oleaginous yeasts grow in the residual liquid left after ethanol separation with, again, no added nutrients? In order to have a base for comparison, ethanol fermentations were first also carried out using clarified only sweet sorghum juice.

2. Materials and methods

2.1. Sweet sorghum juice

The juice used had been kept frozen at −15°C for 16 months. It had a pH of 5.45, 11.4°Brix and 10.9 g/100 mL. It was obtained by pressing freshly cut stalks of M81-E sweet sorghum variety, employing a stainless steel pilot three roll crushing mill (Vencedora Maqtron Model 721) with a 2-HP motor. The M81-E sweet sorghum variety has been shown to grow well in semi-tropical and tropical conditions; also, three crops per year can be harvested if it seems appropriate [3,25].

2.2. Juice clarification

Clarification was done in several batches and every time it was performed by adding slowly with constant agitation a previously prepared calcium oxide suspension (2.4 g/20 mL, Merck reagent 1.02106.0500) to 300 mL at 80–85°C of sweet sorghum juice until a pH of 7.0 was attained [26]. The beakers were left cooling overnight and then, solids were decanted. The separated liquids were further polished by centrifugation at 2000*g* for 5 min at 10°C (Sorvall RT7 refrigerated centrifuge) and the pH adjusted to 5.00 with diluted sulfuric acid. The clarified material was pooled and kept under refrigeration at 5°C until further use.

2.3. Juice concentration

Water evaporation from clarified juice was done in a rotary evaporator operated batch wise under vacuum (Rotavapor RE 121 Büchi) until the initial liquid volume had been reduced to about half.

2.4. Microorganisms and inoculum preparation

Three *Saccharomyces cerevisae* yeasts were tested for ethanol production, CBS 381 and CBS 400 (Centraalbureau voor Schimmelcultures, Utrecht), and a local strain, PAN, currently used by distilleries in our country. Four oleaginous yeasts were employed in the process second step: *Trichosporon oleaginosus* DSMZ 11815 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig), *Rhodotorula glutinis var. glutinis* CBS322, *Lipomyces starkeyi* CBS 1807, and *Yarrowia lipolytica* CBS 2075.

The inoculum for all yeasts was prepared as follows: a pure culture sample was grown in a 30 g/L Sabouraud broth (Merck, 2% glucose, 0.5% animal peptone, and 0.5% casein peptone) plus 1% additional sucrose. One hundred and twenty-five milliliters of broth was added into a 250 mL flask, sterilized at 121°C for 20 min, cooled, inoculated, and agitated at 250 rpm at 30°C for 48 h (Incubator Shaker Lab Companion Model SI-600). The suspension was centrifuged at 1600*g* for 5 min at 10°C (Eppendorf Table-top Refrigerated Centrifuge Model 5804R). The solid pellet was suspended in deionized water and the optical density adjusted approximately to value of 1.8.

2.5. Ethanol production

Experiments were done with clarified juice and with clarified and concentrated juice in order to visualize the individual sugars uptake under the two different fermentation conditions.

2.5.1. Ethanol production with clarified juice

100 mL of the clarified sweet sorghum juice was placed in 250 mL Erlenmeyer flasks and 10 mL of the yeast inoculum was added. The flasks were kept for 72 h at 30°C under static conditions. The ethanol producing yeast was separated by centrifugation at 2000*g* for 5 min at

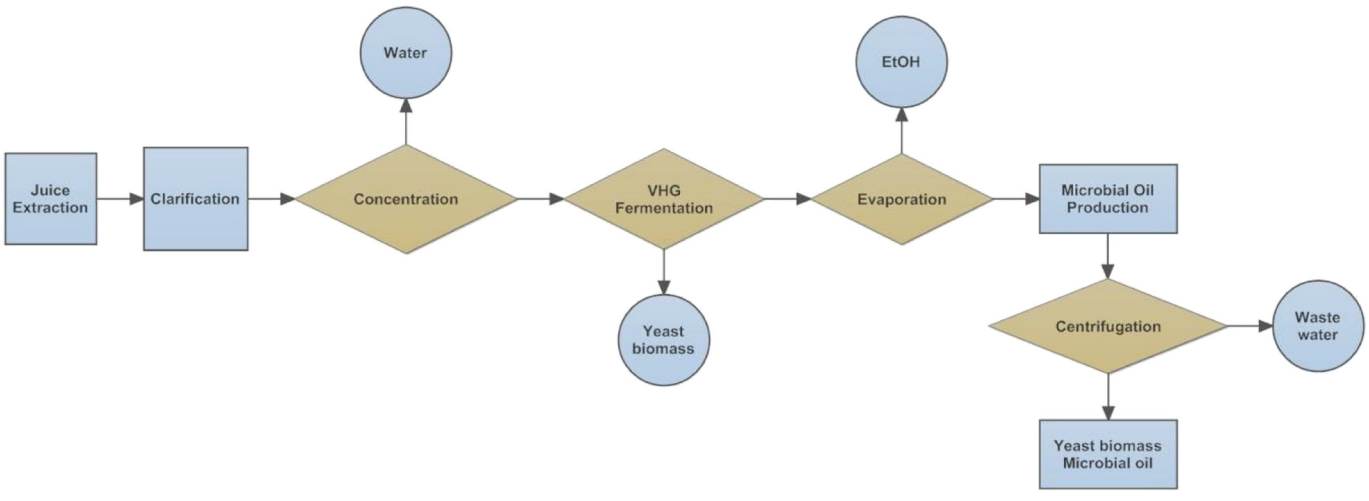

Fig. 1. Block diagram of the two step process for ethanol and microbial oil production from sweet sorghum juice.

Fig. 2. Original M81-E sweet sorghum juice (left side as observed by reader) and clarified and centrifuged juice (right side).

10°C (Sorvall RT7 refrigerated centrifuge) and a liquid sample was sent for sugar and ethanol analysis as explained below.

2.5.2. Ethanol production with clarified and concentrated juice

The flasks with 100 mL of the clarified and concentrated sweet sorghum juice were placed as follows: one flask in static conditions at 30°C for 96 h, and another flask in agitated conditions for 72 h at 30°C and 120 rpm (Incubator Shaker Lab Companion Model SI-600). The reason for the difference in fermentation periods was that in the agitated flasks no more CO_2 evolution occurred at 72 h and the static flasks reached that stage at 96 h. In both cases, the ethanol producing yeast was separated by centrifugation at 2000*g* for 5 min at 10°C (Sorvall RT7 refrigerated centrifuge) and a liquid sample was sent for sugar and ethanol analysis as explained below.

2.6. Ethanol removal from fermentation liquor

All remaining liquids from the VHG ethanol fermentations were placed in a 500 mL flask, and heated to evaporate ethanol in a Soxhlet apparatus. Ethanol was not quantified in the remaining liquid after evaporation.

2.7. Microbial oil production

100 mL of the residual liquid from the ethanol separation step was placed in 250 mL Erlenmeyer flasks and 10 mL of the yeast inoculum was added. The flasks were agitated at 250 rpm at 30°C for 7 d (Incubator Shaker Lab Companion Model SI-600). Yeast dry weight and total sugars were determined as explained below. Yeast biomass was recuperated by centrifugation at 1600*g* for 5 min at 10°C (Eppendorf Table-top Refrigerated Centrifuge Model 5804R). It was kept frozen for two days at −10°C. It was thawed, placed on a glass surface and dried with air at 65°C. Moisture and oil content were determined as explained below. The yeast oil accumulation with time was not followed.

2.8. Analytical procedures

Sugars in the filtrate were determined with an Agilent 1100 high pressure liquid chromatograph, an Agilent 1200 refractive index detector, a Zorbax NH2, 25 cm long, 4.6 mm internal diameter column, employing acetonitrile in water (70–30), as the solvent phase. Ethanol was quantified employing an Agilent 6890 N gas chromatograph, with an HP-Plot/Q, 30 m long, 32 mm internal diameter column. Moisture of the dry yeast biomass was determined gravimetrically by placing a sample in an oven at 65°C until constant weight. The oil content in the dry biomass was determined employing the Folch solvent with some modifications [27]. Briefly, the procedure was as follows: a known weight of yeast, close to 25 mg, was placed in a 15 mL tube. Six milliliters of the Folch solvent was added (2:1 $CHCL_3$:MeOH) and the contents were agitated on a Vortex Mixer for 5 min. The tube was left for 24 h at ambient temperature. The contents were centrifuged at 1600*g* for 5 min at 10°C (Eppendorf Table-top Refrigerated Centrifuge Model 5804R). The upper layer was discarded and the lower layer was filtrated employing Whatman paper No.1 into a small beaker of known weight. The beaker was placed in an oven at 65°C for 24 h. It was cooled and weighed.

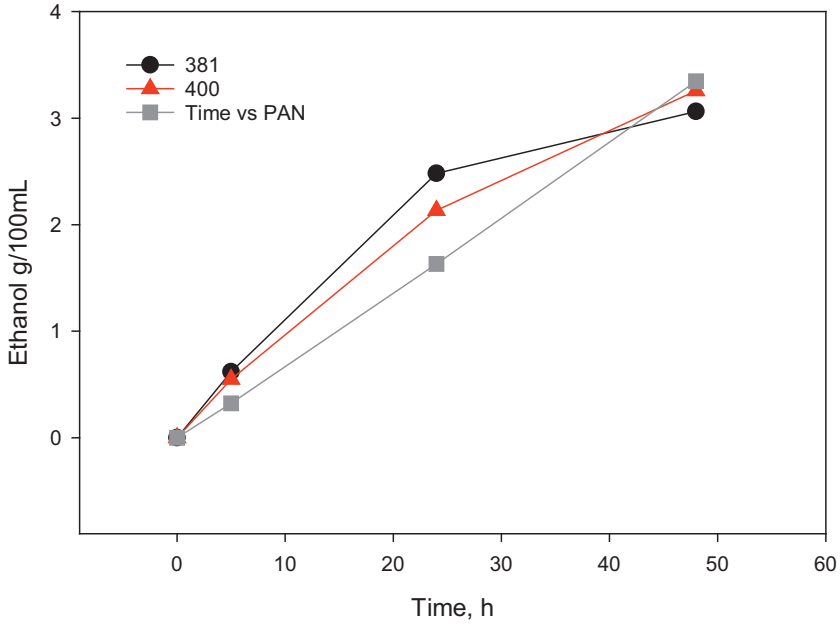

Fig. 3. Ethanol production by all yeasts from clarified sweet sorghum juice as a function of batch time.

Table 1
Ethanol batch productivity in g/L·h^{-1}, total sugar consumption as % of original value, and ethanol yield in g ethanol/g sugar consumed, after 48 h batch fermentation employing clarified sweet sorghum juice.

Yeast	Ethanol batch productivity	Total sugar consumption	Ethanol yield
381	0.64	93	0.39
400	0.68	96	0.45
PAN	0.70	90	0.45

3. Results and discussion

3.1. Juice sugar distribution

The individual sugar distribution of the frozen juice was sucrose 62%, glucose 21% and fructose 17%. The sugar distribution was quite similar to those we reported previously for fresh extracted juice for this sweet sorghum variety planted at the same site, which was on average: 65–68% sucrose, 17–22% glucose and 12–15% fructose [3,25]. This fact indicates that the frozen juice long storage time had a negligible effect upon the sugar distribution. On the other hand, several researchers have reported different sugar distribution data for M81-E planted in different growing regions where a wide range of values have been obtained: 48–80% sucrose, 10–28% glucose and 10–23% fructose [6,8,28,29]. Such differences in sugar distribution might be attributable to different environmental parameters, managing practices and soil properties.

3.2. Juice clarification

Clarification produced a juice with an increased transparency as can be seen in Fig. 2. The clarified juice pH was adjusted to 5.0, and it had 9.6°Brix and 9.4 g/100 mL of total sugars. The individual sugar composition was: 60% sucrose, 22% glucose and 18% fructose. There were no significant differences in the distribution of individual sugars between the original juice and the clarified one, which coincides with previous similar results for M81-E [28].

3.3. Clarified sweet sorghum juice experiments

The ethanol production profiles as a function of time are shown in Fig. 3. The final ethanol values after 48 h were in the range of 3.1 to 3.3 g/100 mL. Ethanol batch productivity, total sugar consumption and ethanol yield data are shown in Table 1. The results for all these parameters show that the three yeasts performed in a rather similar fashion. Ethanol yield was acceptable for 400 and PAN, but it was low for 381. The ethanol productivity data reported in Table 1 is slightly above the one previously informed for M81-E extracted juice by Dávila-Gómez et al. [30] of 0.59 g/L·h^{-1}, however, is below the higher value reported by Guigou et al. [31] of 1.92 g/L·h^{-1} for extracted M81-E juice enriched with inorganic nitrogen, phosphorus and magnesium salts. Ethanol productivity data for extracted juice from other sweet sorghum varieties and hybrids are common in the literature and they expand in a wide range of values, for example, a value of 2.04 g/L·h^{-1} has been reported [32] for sweet sorghum 18°Brix juice supplemented with ammonium sulfate. Juice enrichment has been a common practice; however, such practice increases raw material cost and causes more problems in waste treatment systems. Sugar consumption was normal and the residual sugar as can be observed in the table was between 4 and 10% of the original value.

The individual sugar uptake profiles as a function of time are shown in Fig. 4. In all yeasts, sucrose was continuously consumed and glucose and fructose showed an early increase in the fermentation and then were consumed. The increase was caused by the extracellular sucrose hydrolysis by yeast invertase. However, in 381 and 400, both glucose and fructose after 5 h were consumed by the cell at about the same rate, and in PAN fructose uptake took place after 25 h, when sucrose and glucose concentrations were low enough. Individual sugar consumption by all yeasts was above 94%, with the exception of fructose by PAN.

Most of the data published on sweet sorghum fermentations shows incomplete sugar utilization. Some authors indicated that residual sugars were mainly reducing sugars [28]. Other authors have pointed out that fructose is the reducing sugar not utilized [33]. Kundiyana et al. [34] tested two commercial yeasts at ambient temperature fermentation of sweet sorghum juice with urea addition and pH adjustment, in which, one of the yeasts, consumed all the sugar

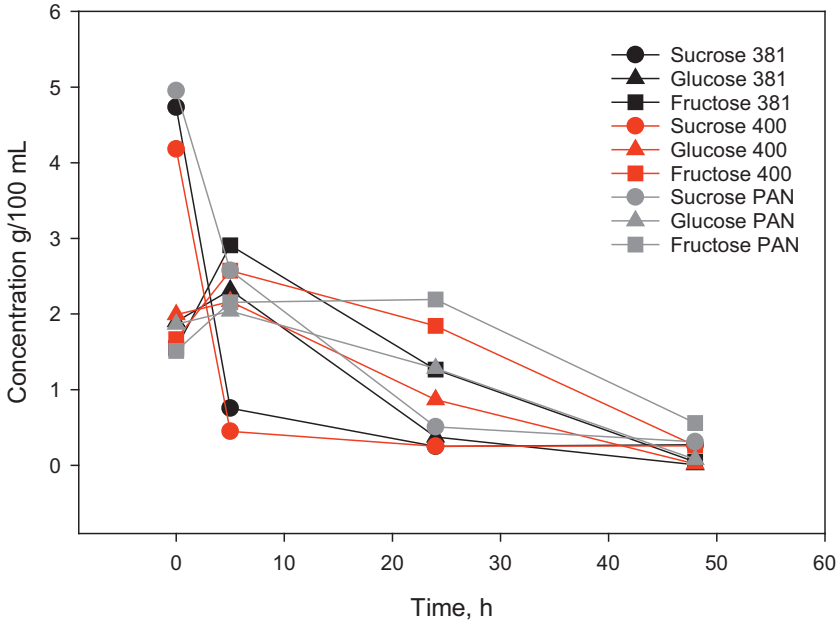

Fig. 4. Individual sugar uptake by all yeasts in clarified sweet sorghum juice as a function of batch time.

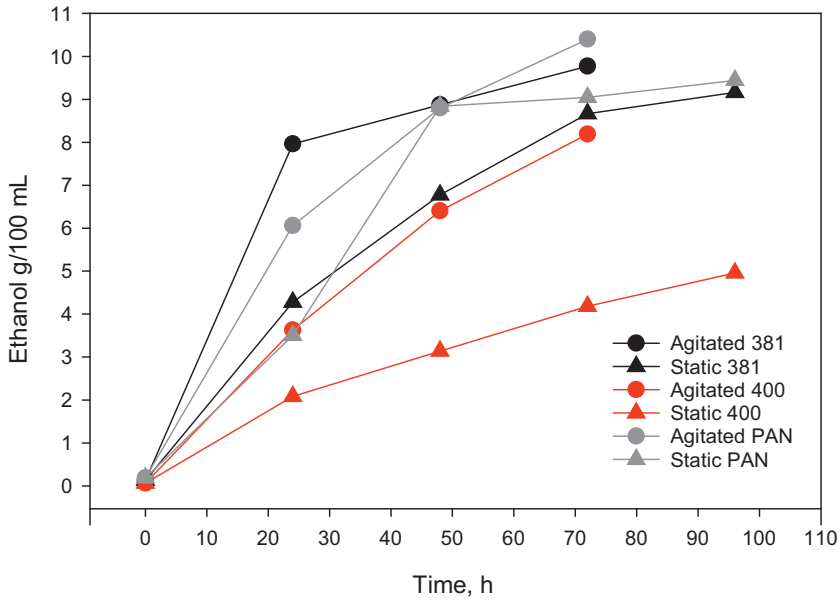

Fig. 5. Ethanol production by all yeasts from clarified and concentrated sweet sorghum juice as a function of batch time in agitated and static flasks.

available in 120 h with a product yield of 0.49 g of ethanol per g of sugar consumed.

In general terms, it is well known that in initial mixtures of sucrose, glucose and fructose, glucose is a preferred substrate for common ethanol producing yeasts over sucrose and fructose and that sucrose is hydrolyzed by invertase in the yeast cell membrane into glucose and fructose which are then transported inside the cell [35]. *S. cerevisae* strains possess different glucose transporters of the Hxt family [35] hence its preference as a carbon substrate. in contrast, in *Saccharomyces bayanum* a specific fructose transporter is present [36], and in the osmotolerant *Zygosaccharomyces rouxii* fructose is consumed faster than glucose [37,38]. The ethanol fermentation of dates offer a pertinent case on this respect, as soluble sugars in dates are about 50% fructose, the rest being glucose and small amounts of sucrose. It has been shown with this raw material that *S. cerevisae* strains produce ethanol from glucose and sucrose leaving fructose behind, a process with two final products, ethanol and fructose [39, 40]. These *S. cerevisae* are hexokinases-less strains which have been shown to produce ethanol and fructose from pure sucrose [41].

3.4. Ethanol production from clarified and concentrated sweet sorghum juice

The concentrated and clarified sweet sorghum juice had a pH of 5.0, 23.8°Brix and 23.7 g/100 mL of total sugars. Total sugars were 2.5 times more than the original clarified juice. The individual sugar composition was: 60% sucrose, 23% glucose and 17% fructose, quite similar to the original clarified juice. This result confirmed that there was no sucrose inversion to glucose and fructose or losses to thermal degradation during water evaporation. The ethanol production profiles are shown in Fig. 5. The profiles were quite different from those obtained with the original clarified juice (Fig. 3). First, significant different patterns were shown by yeasts, and second, as expected due to the higher initial sugar concentration, fermentation took more time. As can be seen, ethanol concentrations for 400 as a function of fermentation time, either in static or agitated flasks, were the lowest among the three yeasts. Also, agitated flasks produced more ethanol than those in static conditions for all the yeasts tested. Aeration provided by agitation has been shown previously to be necessary in VHG sweet sorghum fermentations in order to improve yeast ethanol tolerance [42]. It has been known, also, that aeration reduces ethanol inhibition for yeast growth and glycerol production [43] hence increasing ethanol yield [44,45]. The higher ethanol concentrations achieved were produced by 381 and PAN in agitated flasks, a figure close to 10 g of ethanol/100 mL.

Ethanol batch productivity, total sugar consumption and ethanol yield data are shown in Table 2. Agitation caused an increase in all of these parameters and 381 and PAN were far superior to 400. The ethanol productivity data for agitated flasks is below the range of some values reported in the literature for concentrated sweet sorghum juice without added nutrients, 1.96–2.84 g/L·h^{-1} [12,46]. Almost all results published in the literature for such VHG conditions have enriched the concentrated juice, looking for an increase in ethanol productivity, with inorganic nitrogen and minerals, or organic nitrogen present in yeast extract or dried spent yeast. The ethanol productivity values reported were increased to close to 3.5 g/L·h^{-1} with practically total sugar utilization [47,48,49,50]. An interesting approach in such efforts was the simultaneous fermentation of previously mashed and enzyme treated sweet sorghum grains and concentrated sweet sorghum juice [51,52,53]; the mashed grains providing the necessary organic nitrogen and other grain nutrients.

The uptake profiles of individual sugars for all yeasts as a function of time are shown in Fig. 6 for fermentation in agitated flasks and in Fig. 7 in static flasks. The uptake profiles for agitated flasks showed that sucrose and glucose were continuously consumed by all yeasts; fructose, on the other hand, showed for 381 and 400, an early increase and then was only partially consumed, however, for PAN it was consumed continuously. This behavior was different to the one obtained with normal clarified juice as shown in Fig. 4. It is quite

Table 2
Ethanol batch productivity in g/L·h^{-1}, total sugar consumption as % of original value, and ethanol yield in g ethanol/g sugar consumed, after 72 h batch fermentation in agitated flasks, and after 96 h in static flasks, employing clarified and concentrated sweet sorghum juice.

Yeast	Flask	Ethanol batch productivity	Total sugar consumption	Ethanol yield
381	Static	0.93	85	0.44
381	Agitated	1.36	86	0.50
400	Static	0.51	37	0.38
400	Agitated	1.14	80	0.44
PAN	Static	0.96	91	0.43
PAN	Agitated	1.44	90	0.50

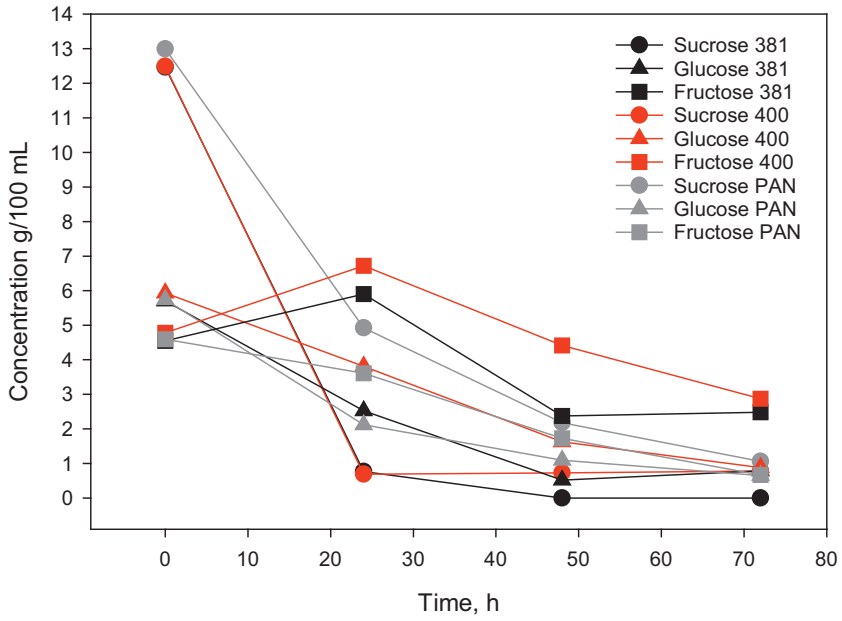

Fig. 6. Individual sugar uptake by all yeasts in clarified and concentrated sweet sorghum juice as a function of batch time in agitated flasks.

possible then, that agitation, providing oxygen to the media, caused the differences. We could not find in the published literature individual sugars uptake data under VHG conditions employing sweet sorghum juice in order to compare with our own data. Previous authors have sought to optimize VHG conditions looking for total sugar consumption by the yeast cell. However, in VHG dates fermentation, rich in fructose and glucose and poor in sucrose, glucose was continuously metabolized, the rather small amount of sucrose was utilized until glucose concentration in the medium was low, and fructose consumption was only a small amount [39,40].

It seems then, that how and to what extent individual sugars utilization takes place from an initial mixture of sucrose, glucose and fructose in raw materials like sweet sorghum juice or dates, will depend mainly on the yeast strain employed, the initial sugar content, the nutrients added, with emphasis on the nitrogen source, and the fermentation time.

In summary, we believe that PAN gave the best overall response during VHG fermentation: producing a relative high ethanol productivity and sugar consumption, leaving a residual liquid phase, after yeast biomass and ethanol separations, with higher fructose content in relation to glucose and sucrose for the next process step.

3.5. Oleaginous yeast growth in pooled residual liquid

The pooled residual liquid from the ethanol distillation had a pH of 4.3 and contained 7.08 g/mL of total carbohydrates, with the following individual sugar distribution: 52% fructose, 25% sucrose and 23% glucose. The experimental data for dry biomass, total sugar consumption, biomass yield and oil content, and oil productivity are listed for the four oleaginous yeasts in Table 3. The dry biomass data was within the order of magnitude of previous results employing different yeasts growing in sugarcane molasses and sweet sorghum

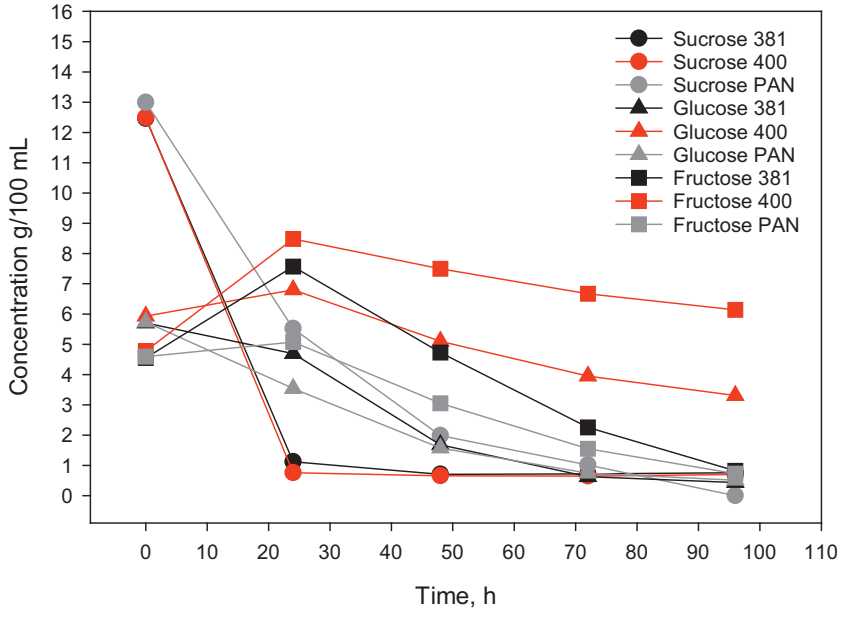

Fig. 7. Individual sugar uptake by all yeasts in clarified and concentrated sweet sorghum juice as a function of batch time in static flasks.

Table 3
Dry biomass produced in gL^{-1}, total sugars consumption as % of initial value, yield expressed as dry biomass divided by sugars consumed, dry biomass oil content as %, oil in flask as gL^{-1}, and oil productivity as g of oil L^{-1} d L^{-1} of oleaginous yeasts grown in pooled residual liquid of previously ethanol fermented clarified and concentrated sweet sorghum juice after 7 d in agitated flasks.

Yeast	Dry biomass	Total sugars consumption % original	Yield	Dry biomass oil	Oil in flask	Oil productivity
T. oleaginosus	24.43	81.21	0.12	15.89	3.88	0.55
R. glutinis	19.21	59.38	0.22	13.69	2.66	0.38
L. starkeyi	24.99	79.27	0.14	14.07	3.51	0.50
Y. lipolytica	19.15	54.81	0.19	16.18	3.09	0.44

juice [54,55,56,57]. The oil in the flask and the oil productivity data, however, were in the low range of values, because the dry biomass oil content was relatively low. The three yeasts tested gave rather similar results, with exception of total sugars consumed, as *T. oleaginosus* and *L. starkeyi* consumed around 80% of the sugars present, especially fructose which was totalled consumed. *R. glutinis* consumed fructose and glucose preferentially. *Y. lipolytica* consumed the three sugars approximately in equal parts. Sitepu et al. [58] reported experimental data on different carbon sources by a large group of oleaginous yeasts, however, fructose was not included, nor the yeast *T. oleaginosum*. Nevertheless, our sugar consumption results coincide partially. Sitepu et al. [58] obtained delayed growth for *L. starkeyi* in sucrose and glucose and in glucose for *R. glutinis*, which coincides with our data. However, no growth was reported for *Y. lipolytica* on sucrose; Vieira et al. [57] also reported no growth of *Y. lipolytica* on molasses. These results are contradictory with our data as we observed about 60% sucrose conversion with the *Yarrowia* strain tested.

Three factors might have been responsible for the relatively low dry biomass oil content: incomplete sugar uptake, nitrogen availability and pH. It seems that seven days were not enough time for yeasts to consume all sugars. Indeed, some authors recommend nine days [14]. We had obtained previously with *T. oleaginosus* a cell biomass with a 28% oil content, and an oil productivity of 0.86 g of oil L^{-1} d^{-1} in seven days [24]. However, concentrated sweet sorghum juice had been enriched with inorganic nitrogen before the VHG ethanol fermentation and we had estimated that the C/N ratio at the beginning of the oleaginous yeast growth was 86, close to the recommended figure of 100 [14]. In this work the total nitrogen concentration at the start of the oleaginous yeast growth was not measured, however it can be estimated. Organic nitrogen consumption in sweet sorghum juice ethanol fermentation has been reported to be in the order of 71% [12], which translates into a C/N ratio of 42 at the start of the oleaginous yeast growth, figure that is about 50% of the optimum value. Hence, future experiments should consider extending the oleaginous yeast growth time and the addition of organic nitrogen sources, for example, the *S. cerevisae* biomass discarded in the ethanol fermentation step previous cell hydrolysis, as suggested by Suwanapong et al. [59].

An acid pH effect on the growth and lipid accumulation phases of oleaginous yeasts is still under debate as different strains have showed specific behavior. For example, the early work of Kessell [60] showed that low pH values retarded growth of *Rhodotorula gracilis* but the lipid production rate increased, although the final lipid concentration did not change. Johnson et al. [61] for the same yeast found that lipid accumulation was strongly affected by the medium pH when fed-batch on glucose and the maximum lipid yield was obtained at pH 4.0. Vieria et al. [57,62] fixed pH at 4.8 for their growth experiments on molasses. Naganuma et al. [63] grew *L. starkeyi* on glucose and showed that the cultural temperature and the initial pH value of the medium affected the total cell number and lipid content; the optimum pH value was 4.9. These results were similar to the ones by Angerbauer et al. [64] which reported for the same yeast grown on sewage sludge, that the highest lipid accumulation was found at pH 5.0. Shen et al. [65] found for *Trichosporon fermentans* in a molasses medium acceptable growth and lipid accumulation for pH 3.0, 5.0 and 7.0. Zhu et al. [55] employing *T. fermentans* on glucose or molasses found that growth and lipid accumulation could be achieved at pH 4.5, 5.0 and 5.5, the results were slightly lower than those obtained at pH 6.0–6.5. *Y. lipolytica* grows adequately in a wide pH range, 5.0 to 7.0 [66]. In summary then, experimental evidence demonstrates that an acid pH for growth and lipid accumulation is not inhibitory for growth, nor for lipid accumulation, however, it would be wise to optimize for pH once an oleaginous yeast is selected for a specific substrate.

4. Conclusions

In this study several yeasts were compared in the first and second steps of our proposed process which consists of ethanol fermentation of clarified and concentrated sweet sorghum juice by ethanol producing yeasts, followed by microbial oil production by oleaginous yeasts employing the carbon and nitrogen in the residual liquid phase after removal of the ethanol producing yeast biomass by centrifugation and the ethanol product by evaporation. We found that PAN gave the best overall response during VHG ethanol fermentation over the two other yeasts tested; relative high ethanol productivity, 1.44 g ethanol/L·h^{-1}, and 90% of sugar consumption. Fermentation media aeration caused by flask agitation during the VGH fermentation produced superior results than static flasks for all yeasts. PAN showed similar individual sugar uptake profiles during fermentation of original clarified juice and in VHG conditions. Sucrose, glucose and fructose were consumed in a continuous manner during 72 h VHG fermentation reaching consumptions of 92, 85 and 82% of the original values. The other two yeasts showed a different pattern of sugar uptake, in which glucose and fructose concentrations increased in the early part of the fermentation due to sucrose hydrolysis, and then were partially consumed. The sugar composition of the remaining liquid after the VHG fermentation, after yeast biomass centrifugation and ethanol evaporation, was different from the composition of the clarified and concentrated sweet sorghum juice. Indeed, fructose was the predominant carbohydrate and sucrose and glucose followed in about the same proportion. In the second step, *T. oleaginosus* and *L. starkeyi* produced higher dry biomass, total sugar consumption and oil productivity than the other two oleaginous yeasts tested; with values around of 25 g/L, 80%, and 0.55 g oil/L·h^{-1} respectively. However, the biomass oil content in all yeasts, which was in the range of 14 to 16%, was low.

Conflict of interest

The authors declare no conflict of interest.

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