

# Valorization of residual soft drinks by baker's yeast production and insight for dairy wastewater whey incorporation

Mariam Kasmi, Amjad Kallel, Lobna Elleuch, Moktar Hamdi and Ismail Trabelsi

## ABSTRACT

Residuals are responsible for the polluting load increase of soft drink industry wastewater due to their high sugar contents. The present work proposes an upstream segregation of residuals to be biologically treated by the bioconversion of their carbohydrates content into baker's yeast biomass. Carbonated soft drinks (CSD) and nectars and juices (NJ) ranges were considered. Different incorporation ratios of NJ in the CSD (0–75%) have been investigated for balanced growth medium. Despite the nitrogen deficiency of media, results showed that NJ incorporation promoted the microbial growth. Media containing more than 50% of NJ exhibited ~25% sugar-biomass conversion rates. The chemical oxygen demand (COD) of the media exceeded 70% at the end of fermentation. Moreover, valuable components were recovered by yeast production. Nutrient consumption rates varied from 65.4% for sugar and calcium content to in excess of 99% for protein and other minerals. In order to investigate an available and low-cost source of nitrogen for yeast production, partial substitution of the soft drink growth medium by bactofugate whey was evaluated. The soft drink-whey mixture medium fermentation resulted in 63% COD removal rate after 28 h. Meanwhile, the biomass production yield revealed an improvement of about 25% compared to the balanced soft drink medium (NJ50).

**Key words** | baker's yeast, COD removal, fermentation, recovery, soft drinks, wastewater, whey

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## INTRODUCTION

Soft drink industry records huge tonnages of residual products, discarded either during the manufacture process or from the distribution chain, or even returned back from the final consumer (Kasmi *et al.* 2016). In general, industries suffer inadequate handling of residual products. Residuals are usually considered as rejects and they are meant to join the industry's wastewater. Nevertheless, residuals contribute to the increase in the organic load of the plant wastewater due to their high sugar content. Furthermore, their discharge to public sewer is strictly regulated by law and no longer accepted. In this context, biological processes as ways of promoting both treatment and valorization of organic matter containing rejects have gained more and more attention in the last decades (Kasmi 2017). In previous works, carbohydrates from different sources were used to produce valuable products. For hydrogen production,

pineapple waste (Wang *et al.* 2006), and vegetable and fruit processing wastes (Stabnikova *et al.* 2005) were investigated. For yeast biomass production, molasses, mixtures of sucrose and maltose (Mwesigye & Barford 1996), cellulosic biomass hydrolyzates (Yuan *et al.* 2011), bamboo wastewater (Li *et al.* 2009) and whey (Gana & Touzi 2001; Koutinas *et al.* 2009) were used. Moreover, different carbohydrate sources were fermented as substrate for ethanol production, such as juices (Duarte *et al.* 2010), molasses (Siqueira *et al.* 2008), sugarcane residue (Dawson & Boopathy 2007) and musts of dates (Yuan *et al.* 2012).

Over the past years, considerable efforts have been made to discover a new molasses substitute. In this context, musts of dates were investigated for baker's yeast production (Acourène *et al.* 2007; Al-Eid *et al.* 2010), whereas soft drinks present an easily fermentable matrix containing

mainly sucrose, a suitable source of carbon for baker's yeast. Although soft drinks are often nitrogen-poor, it has been demonstrated that the addition of fruit juice greatly enhances the potential for microbial growth. The low pH value of soft drinks and fruit juices inhibits most bacteria, but leaves yeasts unaffected (Wareing & Davenport 2005). Furthermore, wasted products from soft drinks are raw materials that do not incur production costs (Peixoto et al. 2011). Recent studies investigated soft drinks wastewater for bioethanol (Isla et al. 2013), hydrogen (Peixoto et al. 2011), fatty acids (Vergine et al. 2015) and microbial biomass (Kasmi et al. 2016) production. Yet, authors demonstrated that mainly nitrogen supplementation is of a paramount importance when soft drinks are used as substrate. Consequently, other researches were conducted using a molasses and whey mixture for fermentation as a low-cost molasses substitute for biomass (Ferrari et al. 2001), acids (El Asar 2006) and alcohol (Oda & Nakamura 2009) production.

In the frame of extensive efforts to reduce baker's yeast production costs, and valuable soft drink components recovery, this work aims to evaluate the feasibility of using a balanced carbonated soft drinks (CSD) and juice growth medium as substrate for yeast biomass production. The organic matter degrading potential of the microorganism was evaluated. The use of a soft drinks and bacto-fugate whey mixture to strengthen the nitrogen content of the growth medium was also investigated for the production of baker's yeast.

## MATERIALS AND METHODS

### Inoculum preparation

Commercial *Saccharomyces cerevisiae* yeast strain was reactivated on Sabouraud Broth (HiMedia). The medium was sterilized at 121 °C for 20 min. In aseptic condition, 1 gram of commercial yeast grains was added into tubes containing reactivation medium. Inoculated tubes were incubated for 24 hours at 30 °C. Reactivated strains were maintained in surface streaks on Sabouraud Glucose Agar (HiMedia) with Chloramphenicol pour plates. Strains were incubated for 24 hours at 30 °C. Then, they were conserved in a cold room (4 °C). Transplanting was performed each week to avoid contamination and to maintain strain purity and viability. The seed culture was prepared by transferring a loopful of cells to 30 ml seed culture medium containing: glucose 20 g.L<sup>-1</sup>; KH<sub>2</sub>PO<sub>4</sub> 2.4 g.L<sup>-1</sup>; MgSO<sub>4</sub> 0.2 g.L<sup>-1</sup>;

Urea 2.4 g.L<sup>-1</sup>; yeast extract 2.6 g.L<sup>-1</sup> and grown at 30 °C on orbital incubator shaker ZHICHENG ZHWY-103B at 150 rpm for 18 h.

### Fermentation medium

#### Soft drinks medium formulation

Residual soft drinks were collected from the local market. Model samples were formulated using CSD range and nectars and juices (NJ) range selected among local and international brands. According to a previous work, the use of residual soft drinks for single-cell proteins (SCP) production using *Saccharomyces cerevisiae* revealed no aroma effect. Only the sugar content was decisive for the yeast growth (Kasmi et al. 2016). In this study, a single representative sample of each range was prepared by mixing the selected products at equal volumes. Thus, two ranges of soft drink samples are considered: CSD and NJ samples. In order to enhance the CSD sample nutrient intake, a partial and progressive incorporation of the NJ sample was performed as described in Table 1.

#### Soft drinks and whey mixture medium formulation

Dairy wastewater whey was obtained following the bacto-fugate (B) thermal coagulation and clarification according to the methodology proposed by Kasmi et al. (2015). Bactofugate is a dairy effluent generated by milk processing plants. Bactofugate (B) samples were collected from a regional dairy industry (Centrale Laitière du Cap Bon, Soliman, Nabeul governorate). The plant produces approximately 1,696,744 hL of drinking milk per year (MedTest 2012). According to the industry statistics, an average of 1,200 L of this effluent are generated daily after bacto-fugation as a separated milk (Belouarda 2012). Samples were collected in plastic sterile containers of 20 L and stored at 4 °C. Medium mixture was prepared using the (B) whey and the selected soft drink medium at equal volume. Given the

**Table 1** | Soft drinks growth medium formulation using CSD and NJ ranges

Formulated growth medium	CSD sample	NJ sample
CSD <sub>100</sub>	100%	0%
NJ <sub>25</sub>	75%	25%
NJ <sub>50</sub>	50%	50%
NJ <sub>75</sub>	25%	75%
NJ <sub>100</sub>	0%	100%

inability of baker's yeast to metabolize milk sugar (Ferrari et al. 2001), chemical hydrolysis of lactose was carried out according to the protocol proposed by Guimarães et al. (2010).

## Fermentation process

### Soft drinks fermentation

Soft drinks fermentation was carried out in batches using an Erlenmeyer flask (250 mL) containing 50 mL working volume. The basal culture conditions are: initial pH, 5.0; fermentation time, 24 h; temperature, 30 °C; inoculum size 6% (v/v). The orbital speed of the shaker was fixed at 150 rpm. All experiments were performed in triplicate.

### Soft drinks and whey mixture medium fermentation

Batch fermentation process was performed using a 1-L fermenter with 0.8 L of working volume. The fermenter glass vessel was autoclaved beforehand (15 min at 120 °C). Yeast inoculum was added from a seed culture. The fermentation temperature was maintained at 30 °C by water circulation through the fermenter vessel double wall. Agitation was ensured using a magnetic stirrer. The pH was initially adjusted to 5.0. Air injection was performed by an air pump. Dissolved oxygen content in the wort during fermentation was measured continuously using an oximeter (HI 2400, HANNA Instruments). A pH meter (Consort C860) was connected to pick up instant measurements of the fermentation medium's variation in acidity. Samples were withdrawn aseptically during fermentation for analysis of total sugar, biomass growth, yeast cell viability and yeast free medium chemical oxygen demand (COD). Soft drinks and whey mixture medium fermentation was performed in triplicate.

## Analytical methods

Raw medium characterization was performed by minerals content assessments: phosphorous content was determined by vanadomolybdic reagent titration methods, calcium concentration was determined by EDTA method, magnesium content was determined by the hydrotimetric method; zinc, copper and iron assessments were performed using the atomic adsorption method; chloride content determination was carried out according to the Mohr method (Rodier et al. 2009). Sodium and potassium contents were determined using the flame photometer method.

Ammonium assessment was performed using the colorimetric dosage method. Sugar concentration was quantified using the Bertrand method after hot acid hydrolysis of the total sugar content for soft drinks (Audigié et al. 1984) and after defecation (clarification) for the whey-soft drink mixture (AFNOR 1971). Protein content was obtained by the Kjeldahl nitrogen determination method, using 6.38 protein conversion factor (Rombaut & Dewettinck 2007). At the end of fermentation, the culture was harvested using centrifugation (3,500 rpm for 15 minutes) to provide a clear supernatant that was used for the determination of the residual sugar. For growth kinetic and biomass estimation, broth optical density was measured using a spectrophotometer (Thermo Spectronic UV1 equipped with VISIONlite™ software) at 600 nm with appropriate dilution. Biomass concentration was determined by gravimetric analysis after drying to constant weight. Yeast cell viability was determined using Sabouraud agar pour plates count prepared beforehand using culture suspensions at appropriate dilutions. COD was assessed using the open reflux method (Rodier et al. 2009).

## Statistical analysis

The medium's characteristics were analyzed statistically by one-way analysis of variance (ANOVA). The Student–Newman–Keuls test was applied to determine the least significant differences (LSD) among means at  $P < 0.05$  using IBM® SPSS version 20.0.0 software.

## RESULTS AND DISCUSSION

### Soft drink media characterization

Biochemical composition of the different model samples of CSD and NJ residual soft drinks, in comparison with the nutritional needs of baker's yeast, is summarised in Table 2. Results showed that media present acid pH values ranging from 3.3 to 3.6. Although the *Saccharomyces cerevisiae* strain has the advantage of growing in an acidic medium, in which most bacteria do not develop, a pH value between 4 and 4.5 is optimal for its growth (Acourène et al. 2007; Al-Eid et al. 2010). Therefore, a pH adjustment is required to ensure better metabolic reaction of the microorganism. Sugar contents range from 69 to 97 g.L<sup>-1</sup>. Such contents far exceed the baker's yeast requirements for the promotion of oxidative fermentation and biomass production. In fact, beyond a sugar content of 50 g.L<sup>-1</sup>, the

**Table 2** | Biochemical compositions of the formulated soft drinks media using CSD and NJ product ranges compared to nutrient requirements of the baker's yeast

Components	Soft drinks					Nutrient requirements of baker's yeast (Acourène & Tama 2001)
	CSD <sub>100</sub>	NJ <sub>25</sub>	NJ <sub>50</sub>	NJ <sub>75</sub>	NJ <sub>100</sub>	
pH	3.3	3.4	3.5	3.5	3.6	4–5
Sugar (g.L <sup>-1</sup> )	69.1	75.0	85.6	92.6	96.8	20
Protein (g.L <sup>-1</sup> )	0.0	0.5	0.9	1.4	1.8	25
Calcium (mg.L <sup>-1</sup> )	120	180	280	360	400	1,500
Magnesium (mg.L <sup>-1</sup> )	20	42,5	60	105	120	450
Sodium (mg.L <sup>-1</sup> )	1,840	2,360	2,560	2,840	3,360	–
Chlorides (mg.L <sup>-1</sup> )	156	196	243	267	349	–
Potassium (mg.L <sup>-1</sup> )	1,560	1,700	2,800	3,560	4,560	2,400
Phosphorus (mg.L <sup>-1</sup> )	199	291	387	438	702	2,200–2,300
Zinc (mg.L <sup>-1</sup> )	0.41	0.32	0.20	0.07	0.05	0.4
Copper (mg.L <sup>-1</sup> )	0.03	0.02	0.01	0.001	0.002	0.02
Iron (mg.L <sup>-1</sup> )	0.0	20	41	61	175	1
Manganese (mg.L <sup>-1</sup> )	0.0	0.0	0.2	0.5	0.6	0.7

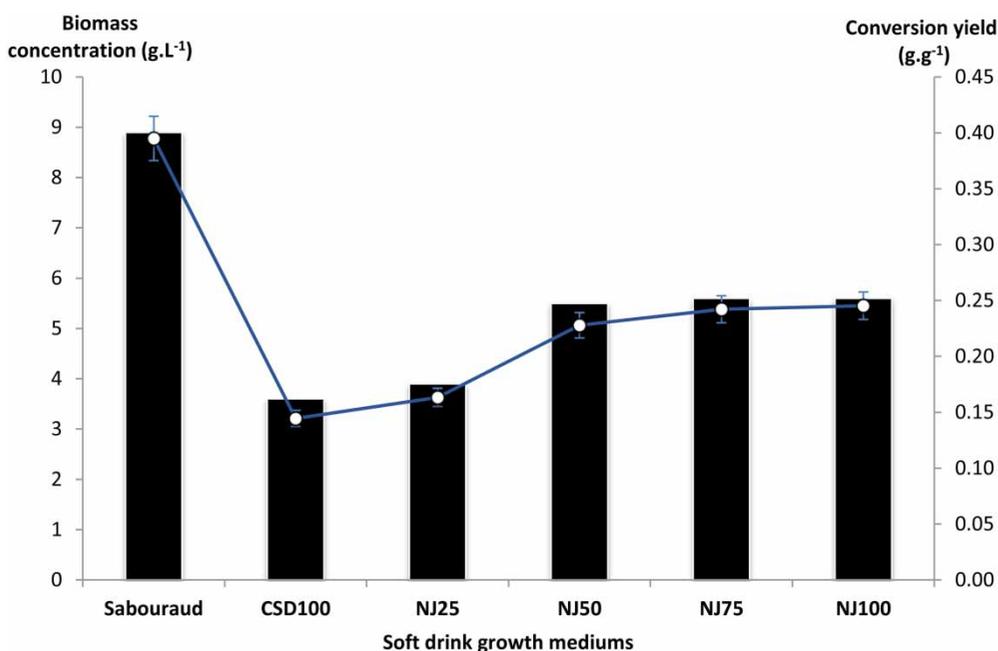
Crabtree effect promoting alcoholic fermentation is evident even in the presence of sufficient amounts of oxygen (Crabtree 1929). Meanwhile, soft drinks exhibit poor protein and phosphorus amounts, although that CSD-NJ mixture has an enhanced nutrient content compared to CSD<sub>100</sub> samples. Calcium content ranging from 120 mg.L<sup>-1</sup> for CSD<sub>100</sub> to 400 mg.L<sup>-1</sup> for NJ<sub>100</sub> does not match the microorganism demand (1,500 mg.L<sup>-1</sup>). The NJ<sub>50</sub>, NJ<sub>75</sub> and NJ<sub>100</sub> samples' potassium contents mostly cover the yeast need (2,400 mg.L<sup>-1</sup>). The CSD<sub>100</sub> sample's zinc (0.41 mg.L<sup>-1</sup>) and copper (0.03 mg.L<sup>-1</sup>) levels are ideally suited to the yeast nutrient needs. The iron intake of NJ samples seems to be beyond the yeast needs. The NJ<sub>100</sub> sample provides 85% of manganese and 26% of magnesium requirements. In addition, chlorides are essential to safeguard the electrolyte balance in the cell. The determined optimum chlorides content for the *Saccharomyces cerevisiae* strain is 285 mg.L<sup>-1</sup> (Mukherjee & Banik 2010). However, soft drink chloride concentrations exceed this value (339–1,240 mg.L<sup>-1</sup>). Such concentrations can act as an inhibitor for yeast growth (Mukherjee & Banik 2010). Sodium content, being also necessary for the microorganism's metabolism, varies between 1.84 and 3.36 g.L<sup>-1</sup> in soft drinks. Meanwhile, high intracellular Na<sup>+</sup> concentration may be toxic for the cell (Sychrova 2004). It has been reported that a sodium concentration of 6.9–11.5 g.L<sup>-1</sup> is able to inhibit the majority of enzymes due to the disruption of the hydrophobic-electrostatic balance between the holding forces of the protein structure. Other metabolic and membrane functions and reactions appear to be affected at such sodium

concentrations (Gaxiola et al. 1992; Gläser et al. 1993). Furthermore, Zimkus et al. (2006) showed that from a concentration of 2.3 g.L<sup>-1</sup> a regression of 15% is noticed in the *S. cerevisiae* metabolic efficiency. Consequently, only CSD<sub>100</sub> and NJ<sub>25</sub> sodium contents comply with the limit value. The NJ<sub>50</sub>, NJ<sub>75</sub> and NJ<sub>100</sub> samples' sodium contents range from 2.5 to 3.4 g.L<sup>-1</sup>, which may induce some metabolic regression in the microorganism's abilities.

In conclusion, the biochemical analysis of the formulated soft drinks media shows an exceptional sugar content. Whereas, syrups dilution is mandatory to avoid the Crabtree effect and the inhibitory effect of some minerals during fermentation. Moreover, an additional protein source is highly recommended for yeast culture using soft drink media.

### Balanced soft drinks medium fermentation

Balanced soft drink media were diluted to adjust the initial sugar content at 20 g.L<sup>-1</sup>. The yeast biomass concentration and the resultant conversion yields after media fermentation are illustrated in Figure 1. A conventional yeast growth medium (Sabouraud broth) was considered as reference. Baker's yeast biomass concentrations in the fermented soft drink media ranged from 3.6 to 5.6 g.L<sup>-1</sup> at the end of the fermentation. The recorded values reveal that the progressive incorporation of NJ syrups in the CSD samples promoted both microbial growth and sugar-biomass conversion yield. In fact, the lower biomass concentration was obtained with the CSD<sub>100</sub> medium. Biomass production



**Figure 1** | *Saccharomyces cerevisiae* strain microbial growth for different soft drinks formulated media: biomass concentration (g.L<sup>-1</sup>) (■) and sugar-biomass conversion yield (g.g<sup>-1</sup>) (○) with NJ syrup incorporation rates.

was improved with NJ<sub>25</sub> (3.9 g.L<sup>-1</sup>) and with NJ<sub>50</sub> (5.5 g.L<sup>-1</sup>). The recorded values reached 5.6 g.L<sup>-1</sup> with NJ<sub>75</sub> and NJ<sub>100</sub>. This improvement was proportional to the conversion yields. CSD<sub>100</sub> conversion yield was 0.14 g.g<sup>-1</sup>. The more NJ syrup is incorporated, the more the conversion yield is important. The recorded conversion yield was 0.23 g.g<sup>-1</sup> with NJ<sub>50</sub>. Yield values stabilized at 0.25 g.g<sup>-1</sup> with NJ<sub>75</sub> and NJ<sub>100</sub>. Compared to the obtained data with molasses growth medium 0.60 g.g<sup>-1</sup> (Moukil 1982), the recorded conversion yields appear to be fairly low. However, they are comparable to Acourène et al. (2007) findings using a dates scrap-based medium for yeast production (0.22 g.g<sup>-1</sup>). Then, it is noteworthy that NJ<sub>50</sub>, NJ<sub>75</sub> and NJ<sub>100</sub> media provided 62–63% of the yeast biomass production obtained with the control medium (Sabouraud). Viable cells count in terms of UFC.mL<sup>-1</sup> revealed good cell viability at the end of fermentation for all culture media. For instance, 3.10<sup>7</sup> UFC.mL<sup>-1</sup> and 5.10<sup>7</sup> UFC.mL<sup>-1</sup> were recorded for CSD<sub>100</sub> and NJ<sub>100</sub>, respectively. The control medium cell viability was assessed at 10<sup>8</sup> UFC.mL<sup>-1</sup>.

These results confirm the CSD medium biomass yield enhancement after NJ syrup incorporation. Therefore, given the cost of the enrichment matrix for an industrial yeast production scale, using NJ syrups to balance the CSD medium nutrients supply would be much more economical and profitable. Accordingly, 50% NJ syrup incorporation in the CSD-based medium gave motivating

results in terms of microbial growth, produced cell viability and registered substrate-biomass conversion yield. Beyond this incorporation level, either for 75% or even 100% NJ medium, no significant improvement was recorded. For this reason, NJ syrup incorporation may be limited to 50% in a CSD medium, resulting in a better nutritional balance for *Saccharomyces cerevisiae* biomass production.

#### Soft drinks fermentation impact on COD removal

Considering the environmental concern, the bioconversion of residuals' sugar content into biomass using *Saccharomyces cerevisiae* was investigated for its organic load removal rates. Figure 2 summarizes COD values before and after the yeast culture, with the evolution of removal rates versus the NJ syrups' incorporation ratios. It is obvious that the more NJ syrups are incorporated, the more the organic loads are important in the raw media. Thus, the initial COD values of the raw media range from 25.0 to 30.3 g.L<sup>-1</sup> for NJ incorporation ratios from 0 to 100%. In fact, the NJ syrup intake in protein, vitamins and minerals contributes to the increase of the organic load of the media. Following fermentation, the yeast-free media recorded a considerable organic load drop. Media COD removal rates range from 43% for CSD<sub>100</sub> to 77% for NJ<sub>75</sub> and NJ<sub>100</sub>. Therefore, the evolution of removal rates confirms the interest of the CSD and NJ syrups mixture, not

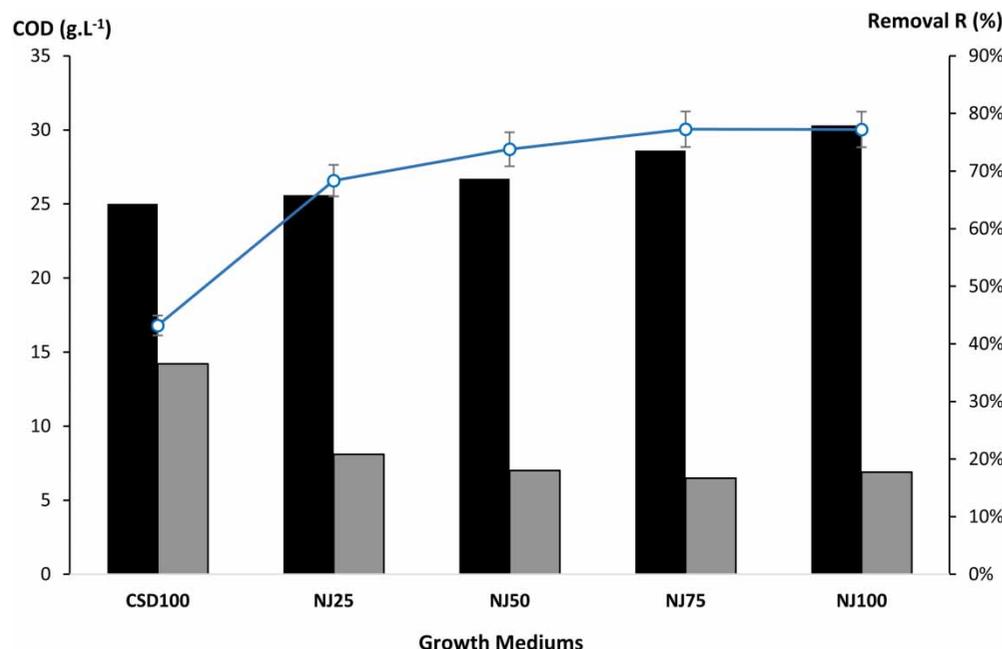


Figure 2 | Organic load reduction in the soft drinks fermented media using baker's yeast: COD initial (■), COD final (▒), COD removal rate R (%) (○).

only for a better biomass yield, but also for a better COD reduction rate in the fermented media. Despite their initial organic matter content being relatively higher than CSD<sub>100</sub> syrup, CSD-enriched media show a noteworthy COD reduction rate improvement from 25% of NJ syrup incorporation (COD removal  $\geq 68\%$ ). Therefore, the CSD<sub>100</sub> medium residual COD value was  $14.2 \text{ g.L}^{-1}$ , while the residual COD values of the yeast-free fermented media containing an NJ fraction varying between 6.5 and  $8.1 \text{ g.L}^{-1}$ . However, a slight increase in the removal rate is detected between NJ<sub>50</sub> and NJ<sub>75</sub> (3%); but no difference is noticed between NJ<sub>75</sub> and NJ<sub>100</sub>. Hence, once again NJ<sub>50</sub> syrup proved to be the optimal medium in terms of the organic load reduction efficiency after fermentation.

In addition to the sugar content consumption, which seems to make the major contribution to the COD value reduction, yeasts have likely profited from the nutritional balance enrichment of protein, vitamins and minerals contained in the NJ fraction. As a part of the study of the recovery efficiency of valuable components from residuals, trace elements analysis after media fermentation using *Saccharomyces cerevisiae* was performed. The obtained results are summarized in Table 3. A decrease in moderate pH values was noticed after media fermentation. A minimum value of 3.5 was reached for the CSD<sub>100</sub> medium. However, no significant difference was recorded between the obtained pH values of the other media at the end of the fermentation. Media sugar content was

initially around  $20 \text{ g.L}^{-1}$ . At the end of fermentation, sugar consumption rates varied between 63% and 68.5%. No significant difference is considered for sugar consumption rates of CSD<sub>100</sub>, NJ<sub>25</sub>, NJ<sub>75</sub> and NJ<sub>100</sub>. However, NJ<sub>50</sub> fermented medium exhibited the lowest residual sugar content ( $6.3 \text{ g.L}^{-1}$ ).

Proteins, initially not available in the CSD<sub>100</sub> medium, were completely metabolized by the yeast in the media where NJ syrups were incorporated. Actually, yeast needs for proteins are far beyond the NJ syrups' protein intake. However, NJ syrup incorporation has certainly improved biomass yields (Peixoto *et al.* 2011; Urbaniec & Grabarczyk 2014). Other minerals also, such as magnesium, zinc, copper, iron and manganese had no detectable traces in the fermented musts. It has been reported that zinc, copper and manganese are essential because of their positive effect on the respiratory activity and the growth rate of *Saccharomyces cerevisiae* (Jones & Gadd 1990). Nevertheless, calcium, potassium and phosphorus contents were not exhausted although that initial nutrient content in the culture medium did not satisfy the yeast's nutritional requirements (Acourène & Tama 2001). In fact, the average consumption rates of these minerals were 34% for calcium, 41% for phosphorous and 57% for potassium. However, the average sodium uptake was evaluated at 50%, although that sodium yeast need was not stated by Acourène & Tama (2001). This supports the reported data about the necessity of sodium to maintain the yeast (Sychrova 2004).

**Table 3** | Biochemical composition of the fermented soft drinks media using *Saccharomyces cerevisiae* and nutrient consumption rate average

Constituents	Soft drinks media					Average consumption rates (%)
	CSD <sub>100</sub>	NJ <sub>25</sub>	NJ <sub>50</sub>	NJ <sub>75</sub>	NJ <sub>100</sub>	
pH	3.5 <sup>a</sup>	3.7 <sup>a</sup>	3.6 <sup>a</sup>	3.6 <sup>a</sup>	3.7 <sup>a</sup>	–
Sugar content (g.L <sup>-1</sup> )	6.8 <sup>a</sup>	7.2 <sup>a</sup>	6.3 <sup>b</sup>	6.9 <sup>a</sup>	7.4 <sup>a</sup>	65.4
Protein (g.L <sup>-1</sup> )	*	n.d	n.d	n.d	n.d	>99
Calcium (mg.L <sup>-1</sup> )	n.d	n.d	20.4 <sup>a</sup>	60.1 <sup>b</sup>	100 <sup>c</sup>	34
Magnesium (mg.L <sup>-1</sup> )	n.d	n.d	n.d	n.d	n.d	>99
Sodium (mg.L <sup>-1</sup> )	236.8 <sup>a</sup>	286.5 <sup>b</sup>	386.0 <sup>c</sup>	429.8 <sup>d</sup>	447.4 <sup>e</sup>	50
Potassium (mg.L <sup>-1</sup> )	90.5 <sup>a</sup>	100 <sup>b</sup>	230 <sup>c</sup>	600 <sup>d</sup>	900 <sup>e</sup>	57
Phosphorus (mg.L <sup>-1</sup> )	42.3 <sup>a</sup>	53.6 <sup>b</sup>	57.7 <sup>b</sup>	76.0 <sup>c</sup>	78.6 <sup>c</sup>	41
Zinc (mg.L <sup>-1</sup> )	n.d	n.d	n.d	n.d	n.d	>99
Copper (mg.L <sup>-1</sup> )	n.d	n.d	n.d	n.d	n.d	>99
Iron (mg.L <sup>-1</sup> )	*	n.d	n.d	n.d	n.d	>99
Manganese (mg.L <sup>-1</sup> )	*	*	n.d	n.d	n.d	>99

n.d: not detectable at threshold of the analysis method used.

\*Component initially undetectable in the medium.

a,b,c,d,e values within the same row followed by different superscripts are significantly different at ( $P \leq 0.05$ ).

In addition, it was reported that the culture medium nitrogen content affects the yeast growth, the growth rate and the fermentation time (Bisson 1999; Bell & Henschke 2005). The required amount of nitrogen for unproblematic fermentation depends on both the medium's composition and the yeast strain used. In fact, *Saccharomyces* strains are known for their wide variability in nitrogen requirements, which would not be attributed only to the fermentation conditions, but also to the strain's genetic background (Barbosa et al. 2009). Nitrogen regulates the formation of by-products and end products. Several studies on the effect of growth media ammonium supplementation on the growth kinetics of the fermentation have been conducted (Mendes-Ferreira et al. 2004). The addition of di-ammonium phosphate (DAP), urea or yeast extract to balance the medium's nitrogen content is highly recommended (Barbosa et al. 2009). In the frame of several efforts to find an available and low-cost source of protein/nitrogen, this work proposes investigating the effect of bactofugate whey incorporation in soft drink growth media for yeast production.

### Soft drinks and bactofugate whey mixture fermentation kinetic

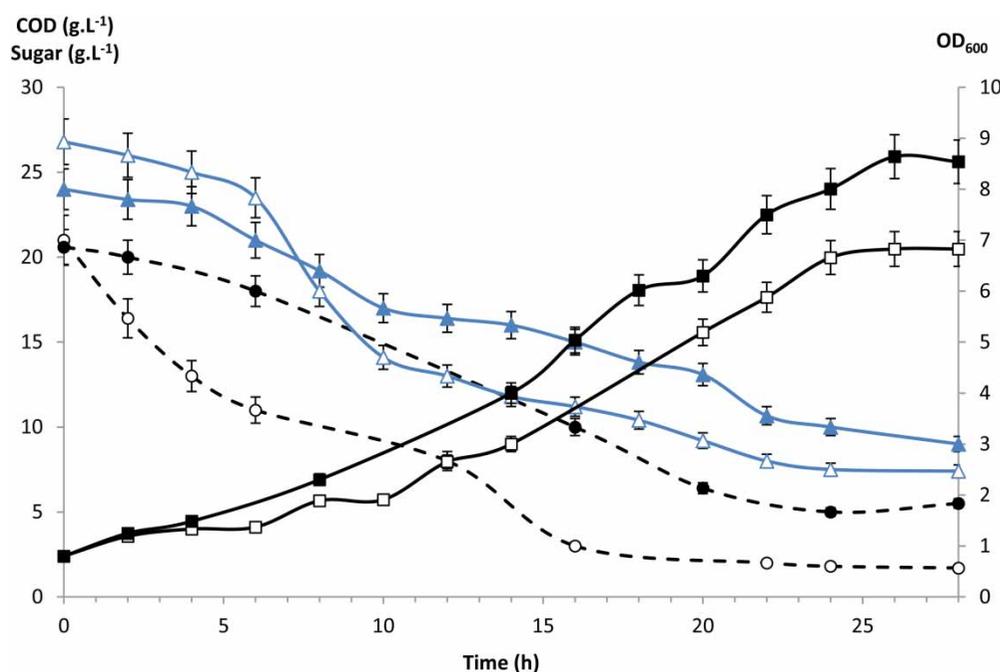
The balanced soft drink medium NJ<sub>50</sub> and the clarified bactofugate whey (B) mixture (NJ<sub>50</sub> + B) was considered as growth medium for *Saccharomyces cerevisiae* production.

Such a medium would be beneficial for the cultivation of yeast given the whey's richness of mineral and nitrogenous matter compared to soft drink syrups (Table 4). Fermentation kinetics of the NJ<sub>50</sub> medium supplemented with B whey (NJ<sub>50</sub> + B) compared to the fermentation kinetics of NJ<sub>50</sub> syrup are illustrated in Figure 3. The evolution of the produced cell density (OD<sub>600</sub>), sugar consumption rates and COD reductions were determined.

The results show that the microbial growth evolution expressed in OD<sub>600</sub> recorded a considerable improvement with the mixture medium NJ<sub>50</sub> + B compared to the microbial growth of yeast with the NJ<sub>50</sub> medium. This improvement was detectable during the first five hours of fermentation. A biomass yield increase of 17% was recorded

**Table 4** | Biochemical composition of the bactofugate whey (B) compared to the estimated nutrient content of NJ<sub>50</sub> medium

Parameters	Bactofugate whey (B)	NJ <sub>50</sub> growth medium
pH	6.4 ± 0.2	6.1 ± 0.2
Sugar (g.L <sup>-1</sup> )	19.8 ± 1.4	21.4 ± 2
Protein (g.L <sup>-1</sup> )	3.7 ± 0.6	0.22 ± 0.0
Calcium (g.L <sup>-1</sup> )	0.3 ± 0.0	0.07 ± 0.0
Magnesium (mg.L <sup>-1</sup> )	20.0 ± 2	15 ± 1
Potassium (g.L <sup>-1</sup> )	0.67 ± 0.02	0.7 ± 0.03
Phosphorus (g.L <sup>-1</sup> )	0.18 ± 0.03	0.96 ± 0.1
COD (g.L <sup>-1</sup> )	20.8 ± 2	26.7 ± 0.9



**Figure 3** |  $NJ_{50}$  and  $NJ_{50} + B$  growth media kinetics during fermentation using *Saccharomyces cerevisiae* in batch mode bioreactor for 28 h: Microbial growth  $OD_{600}$  for  $NJ_{50}$  ( $\square$ ) and  $NJ_{50} + B$  ( $\blacksquare$ ); sugar content ( $g.L^{-1}$ ): for  $NJ_{50}$  ( $\circ$ ) and  $NJ_{50} + B$  ( $\bullet$ ); COD ( $g.L^{-1}$ ) for  $NJ_{50}$  ( $\Delta$ ) and  $NJ_{50} + B$  ( $\blacktriangle$ ).

after 8 hours of fermentation, reaching a maximum of 25% after 14 h. Thereafter, it stabilized at around 20% until the end of fermentation. However, sugar consumption rates were better for the  $NJ_{50}$  medium than for the  $NJ_{50} + B$  medium. In fact, after 6 hours of fermentation the sugar consumption rate for the  $NJ_{50}$  medium reached 47% against only 13% for the mixture medium. After 20 hours of fermentation, the sugar consumption rate for the  $NJ_{50}$  medium had already reached 90% against 69% for the  $NJ_{50} + B$  medium. Towards the end of fermentation, rates stabilized at 92% and 75%, respectively. Sugar consumption amounts were in perfect harmony with the COD removal rates for both media. From the graph, it is clear that the  $NJ_{50}$  medium was initially more organic matter loaded ( $26.7 g.L^{-1}$ ) than the  $NJ_{50} + B$  medium ( $24 g.L^{-1}$ ). After 7 hours of *Saccharomyces cerevisiae* culture on both media, the  $NJ_{50}$  wort started to record smaller COD values than those obtained with the  $NJ_{50} + B$  medium. Yet, the removal rates were 13% and 12%, respectively. After 22 hours of fermentation, the COD removal rate of the  $NJ_{50}$  medium reached 70%. However, the organic load reduction of the  $NJ_{50} + B$  medium was 58%, except that this value continued to improve to reach 63% at the end of fermentation. The COD reduction rate of the  $NJ_{50}$  medium was limited to 72% four hours before the end of fermentation.

Therefore, the enrichment of the NJ syrup using the bacto-fugate whey seems to be advantageous for the yeast

growth yield enhancement. A global increase of 25% in the produced biomass was recorded for 24 h of fermentation. However, an extended fermentation time is necessary to achieve better removal rates with this medium. In fact, the fermentation kinetic of the  $NJ_{50} + B$  mixture medium was characterized by its slowness as regards the sugar consumption rates of the  $NJ_{50}$  medium, which influences the organic load reduction speed of the medium. Besides, it is worth mentioning that the  $NJ_{50} + B$  medium is relatively more complex than the  $NJ_{50}$  medium. Soft drink syrups contain mainly easily fermentable sugar for baker's yeast. In contrast, the mixture medium contains sucrose, milk sugar (lactose) and its derivatives (glucose, galactose, lactulose, etc.) with soluble nitrogenous matter and minerals. Despite the remarkable growth yield of baker's yeast on the mixture medium, some components could not be well assimilated by the microorganism. Actually, the complete hydrolysis of the lactose should be checked to ensure that all the residual sugar components are assimilated by the yeast. Indeed, sugar recovery rates following chemical hydrolysis methods vary between 78% and 99%. In some cases, insoluble solid residues are formed (Feller et al. 1991), which may consist of an inaccessible form of organic material during the fermentation. Thus, enzymatic hydrolysis of milk sugar may be more advantageous for both biomass yield and COD removal rates of the fermented mixture medium. However, considering an industrial biomass

production scale, an economic evaluation would be mandatory to assess whether the potential savings of raw materials and wastewater treatment costs could counterbalance the extra cost of the enzyme purchase (Ferrari et al. 2001).

## CONCLUSION

An upstream segregation of residuals from the soft drink industry was proposed for the bioconversion of their sugar content, as the major factor responsible for their polluting load, into microbial biomass. Soft drinks characterization showed high amounts of sugar content, mainly sucrose, suitable for yeast culture. The progressive incorporation of nectar and juice (NJ) syrups in the CSD media was beneficial for baker's yeast culture. The balanced medium NJ<sub>50</sub>, NJ<sub>75</sub> and NJ<sub>100</sub> exhibited sugar-biomass conversion rates ranging from 0.23 to 0.25 g.g<sup>-1</sup>. Soft drink balanced media provided 62–63% of the microbial biomass production obtained with the control medium (Sabouraud). In addition, good cell viability was obtained at the end of fermentation for all the culture media (10<sup>7</sup> CFU.mL<sup>-1</sup>). Media COD removal rates exceeded 70% by the end of fermentation. Nutrient consumption rates varied from 34% for calcium, 65% for sugar, to in excess of 99% for protein, zinc, copper, iron, magnesium and manganese contents. Partial substitution of soft drink using bacto-fugate whey to strengthen the growth medium nitrogen supply for baker's yeast production revealed an enhancement of about 25% compared to NJ<sub>50</sub>. Meanwhile, the COD removal rate of the soft drink-whey mixture medium was limited to 63% after 28 h of fermentation. Nevertheless, the recorded values might be improved when the fermentation time is extended, since COD values exhibited a slow kinetic evolution compared to the soft drink medium values.

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