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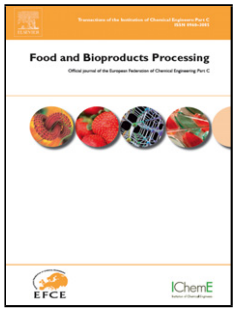


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# Valorisation of fruit and vegetable waste from open markets for the production of 2,3-butanediol

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Highlights

- Fruit and vegetable waste have been used for 2,3-butanediol production
- A screening study showed that *Enterobacter ludwigii* is an efficient 2,3-butanediol producer
- *Enterobacter ludwigii* efficiently produces 2,3-butanediol on fruit and vegetable waste

**Abstract**

The bacterial strain *Enterobacter ludwigii* FMCC 204 was selected as the most efficient 2,3-butanediol (BDO) producer among five strains when cultivated on glucose, fructose, mannose, arabinose, xylose, galactose and sucrose in shake flask cultures. Plums, apples and pears were used for the production of fermentation medium via sequential maceration, suspension in water and centrifugation to collect the supernatant. Sugar production from mixed vegetables was evaluated via sulphuric acid treatment leading to 65.8 % (w/w) of hemicellulose hydrolysis yield at initial solid concentrations of 50 g/L (on a dry basis) treated with 3% (v/v) $H_2SO_4$. Fed-batch cultures of *E. ludwidgii* on fruit derived feedstock resulted in BDO concentration, yield and productivity of 50 g/L, 0.4 g/g and 0.41 g/L/h. BDO production from vegetable waste hydrolysates via fed-batch cultures led to BDO concentration of 17.6 g/L. This study demonstrated that fruit and vegetable wastes from open markets can be used as fermentation feedstocks for BDO production.

**Keywords**: 2,3-Butanediol, *Enterobacter ludwigii*, Fermentation, Fruit and vegetable waste, Open market

## 1. Introduction

In 2014, the worldwide primary production of fresh fruit and vegetables was around 35 million t and 289.8 million t, respectively (Anonymous 2017). In the last five years, Asia holds the major fresh fruit (73.3 %) and vegetable (86.3 %) world production share. In 2014, fresh fruit production in Europe was around 512,000 t, while fresh vegetable production was around 11 million t (Anonymous, 2017). The waste produced during the whole value chain of fruits and vegetables in Europe are distributed in agricultural production (20%), postharvest handling and storage (5%), processing and packaging (2%), distribution (10% for fresh fruits and 2% for processed fruits) and consumption (19% for fresh fruits and 15% for processed fruits) (Gustavsson et al., 2013). In UK households, 25.8% of vegetables, 4.4 % of salads and 16.4 % of fruit are disposed as waste (Ventour, 2008). Open markets generate significant quantities of fruit and vegetable waste, but limited information is available regarding the quantities discarded. The high quantities of wastes produced by the fruit and vegetable value chains necessitate the development of biorefinery concepts for the valorization of non-preventable wastes.

Food supply chain waste can be exploited for biorefinery development including the production of bio-based chemicals and polymers (Lin et al., 2014; Koutinas and Kookos, 2016). Fruit and vegetable wastes contain various free sugars and polysaccharides that can be used as carbon sources for bacterial fermentation. Fruits are rich in glucose, fructose and sucrose that are readily available as a carbon source for microbial bioconversion. Vegetables require a pretreatment step in order to hydrolyse the cellulose and hemicellulose fractions into C5 and C6 sugars. Chandel et al. (2012) reported various dilute acid pretreatment approaches for the hydrolysis of hemicellulose from various agricultural residues and industrial side streams. The low lignin content of vegetable wastes simplifies the hydrolysis of hemicelluloses and cellulose. Dilute acid hydrolysis has been used for the recovery of sugars

from artichoke residues reaching sugar recovery in the liquid fraction of 35.24% (w/w) (del Campo et al., 2006). After the extraction of essential oils and pectin, orange peels were subjected to combined dilute acid pretreatment and enzyme hydrolysis that led to the production of succinic acid with a yield of 0.77 g succinic acid per g consumed total sugars (Patsalou et al., 2017). Weak acids, furan derivatives and phenolic compounds that are toxic to many microorganisms are generated during acid hydrolysis of lignocellulosic biomass (Palmqvist and Hahn-Hagerdal, 2000). Fruits and vegetables are also rich in various value-added components (e.g. bioactive compounds such as polyphenols, pectins) which could be extracted prior to fermentation of the carbohydrate fraction leading to the development of integrated biorefinery concepts (Burniol-Figols et al., 2016; Patsalou et al., 2017).

The production of bio-based 2,3-butanediol (BDO) via fermentation using various renewable resources could lead to the production of an important platform chemical that can be employed for the development of sustainable chemical production, such as 1,3-butadiene and methyl ethyl ketone, or in the production of various end products, such as printing inks, perfumes and fumigants, moistening and softening agent, plasticizers, explosives and food additives (Zheng and Sabra, 2011; Kim et al., 2016). Various bacterial strains, such as *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter aerogenes* and *Bacillus polymyxa* (Celinska and Grajek, 2009, Ji et al., 2011), are able to produce BDO in high fermentation efficiency. Monosaccharide degradation can occur through glycolysis and pentose phosphate pathway depending on the substrate. The key intermediate for BDO and various organic acid production is pyruvate. Pyruvate is initially converted into α-acetolactate through α-acetolactate synthase, which is subsequently converted either directly into acetoin by α-acetolactate decarboxylase or firstly to diacetyl that is subsequently converted into acetoin. The most important pathway for BDO production is the transformation of acetoin into BDO that is catalyzed by acetoin reductase and has a reversible shunt that can result in

extracellular production of BDO or acetoin, usually observed in fermentations when reductive energy is required. There are three different BDO stereoisomers that can be produced via microbial fermentation, namely L-(+)-BDO form (2S,3S-BDO), D-(−)-BDO form (2R,3R-BDO) and meso-BDO form. The mixture of isomers produced and their ratio is dependent mainly on the microbial strain. For instance, *Klebsiella oxytoca* produces the meso-BDO and L-(+)-BDO stereoisomers with the meso form being the main product, while *Paenibacillus polymyxa* produces mainly the D-(−)-BDO isomer (Ji et al., 2011).

BDO can be produced from various crude renewable resources including apple pomace hydrolysate by *Bacillus licheniformis* (Bialkowska et al., 2015), agricultural waste by *Klebsiella* sp. (Wong et al., 2012), Jerusalem artichoke tubers by *Klebsiella pneumoniae* (Sun et al., 2009) and *Paenibacillus polymyxa* (Gao et al., 2010), food waste by *Bacillus amyloliquefaciens* (Sikora et al., 2016) and seaweed hydrolysate by an engineered *E. coli* strain (Mazumdar et al., 2013). To the best of our knowledge, fruit and vegetable waste from open markets have not been evaluated for the production of major platform chemicals, such as BDO. Although individual fruit and vegetable processing side streams generated from the food industry have been evaluated for the production of enzymes, organic acids (e.g. succinic acid, lactic acid) and bio-energy (Panda et al., 2016; Diaz et al., 2017), whole fruit and vegetables from open markets have not been evaluated as potential feedstocks for bio-based platform chemical production considering the high quantities disposed as wastes, the mild pre-treatment required and their high carbohydrate content (Diaz et al., 2017). The use of low cost fermentation feedstocks could lead to significantly reduced BDO production cost (Koutinas et al., 2016). Furthermore, the production of BDO from whole fruit and vegetable waste could be combined with the extraction of value-added co-products (e.g. bioactive compounds, pectins) leading to the development of a sustainable biorefinery.

In the present study, five bacterial strains were initially evaluated for BDO production using the carbon sources contained in whole fruit and vegetable wastes. The bacterial strain *Enterobacter ludwigii* was selected as the most efficient BDO producer. Subsequently, *E. ludwidgii* was used in fed-batch cultures using fruit or vegetable derived fermentation media for the production of BDO.

## 2. Materials and methods

### 2.1 Fruit and vegetable pretreatment

Vegetables and fruits were selected from the central open market in Athens, Greece. The vegetables collected were broccoli (80%), cabbage (5%), lettuce (4%), fresh beans (2%), corn salad and tat soi salad (2%), cabbage (2%), carrots (2%), peppers (red and green) (2%) and eggplants (1%). Broccoli was the main fraction of the vegetables that were collected, thus the pretreatment protocol was developed using broccoli. The broccoli was initially chopped in small pieces and subsequently macerated using a blender. The macerated broccoli was suspended in water to 50 g/L or 100 g/L initial solids concentration (on a dry basis). This suspension was subsequently treated with different $H_2SO_4$ concentrations (0.5%, 1 %, 3 % and 7 % v/v) at 121 $^oC$ for 30 min in order to determine the best combination of sugar release conditions. The conditions that achieved the highest sugar release were also applied to the mixture of macerated and homogenised vegetables collected from the open market. The produced hydrolysate was centrifuged to separate the remaining solids and the supernatant was stored at -20 $^oC$. The mixed sugars (6.08 g/L) contained in the supernatant of optimised mixed vegetable hydrolysate were glucose (0.6 g/L), fructose (0.6 g/L), xylose (0.77 g/L) , galactose (2.46 g/L) and arabinose (1.65 g/L). The mixed vegetable hydrolysate was

concentrated via rotary vacuum evaporation to total sugar concentration of either 15 g/L that was used as fermentation substrate in shake flask and fed-batch bioreactor cultures or 300 g/L that was used as feeding medium in the fed-batch bioreactor fermentation.

The fruits collected were plums, apples and pears. All fruits were macerated and homogenised using a blender. The macerated mixture was diluted with tap water at a solid to liquid ratio of 1:4 and the macerated fruit aqueous suspension was left in the fridge for at least 6 h. The suspension was subsequently centrifuged and the supernatant was stored at -20 $^{o}$C for further use as fermentation substrate. The mixed sugars (around 80.5 g/L) contained in the supernatant derived from the fruits were glucose (32.7 g/L), fructose (44.6 g/L) and sucrose (3.6 g/L). The fruit extract was diluted to initial total sugar concentration of 25 g/L or 50 g/L in the case of shake flask fermentations. In the case of fed-batch bioreactor culture, the fruit extract was diluted to a total sugar fermentation of 28.5 g/L at the beginning of fermentation, while a fraction of the fruit extract was concentrated via rotary vacuum evaporation to a total sugar concentration of 600 g/L that was used as concentrated feeding solution.

### 2.2 Microorganisms

*Enterobacter aerogenes* FMCC 9, *Enterobacter aerogenes* FMCC 10, *Enterobacter ludwigii* FMCC 204, *Enterobacter* sp. FMCC 208 and *Citrobacter freundii* FMCC 207 were isolated from various food products (Drosinos et al., 2005, Doulgeraki et al., 2011). These strains have been identified and characterised in the Department of Food Science and Human Nutrition at the Agricultural University of Athens (Greece) and have been deposited in the culture collection of this department (Drosinos et al., 2005, Doulgeraki et al., 2011, Metsoviti et al., 2012). All strains were stored at -80 $^{o}$C in cryopreservation vials containing liquid pre-culture medium supplemented with 50% (v/v) glycerol.

### 2.3 Pre-culture preparation

Pre-culture (or inoculum) was produced in 500 mL Erlenmeyer flasks with a working volume of 100 mL. The pre-culture medium consisted of glucose (10 g/L), yeast extract (2.5 g/L), peptone (5 g/L), meat extract (5 g/L), $K_2HPO_4$ (2 g/L), $CH_3COONa$ (5 g/L) and $MgSO_4$ (0.41 g/L). The carbon source was sterilised separately from nitrogen and mineral sources. The pre-culture medium was inoculated with a sterile loop from freshly prepared agar plates stored at 4 $^oC$ for maximum one week after preparation. Incubation of inoculum was carried out for 18 h at 30 $^oC$ with 180 rpm agitation.

### 2.4 Shake flask fermentations

Commercial glucose, xylose, galactose, mannose, arabinose and fructose were used as carbon sources in shake flask cultures at initial concentration of around 20 g/L in order to identify the most efficient BDO producing microbial strain. Commercial sucrose was used for the same purpose at initial concentration of around 50 g/L. The fruit extract was used as carbon source in shake flask cultures at two initial total sugar concentrations of 25 g/L and 50 g/L only in the case of *E. ludwigii*. The vegetable hydrolysate was used in shake flask cultures at initial total sugar concentration of 15 g/L only in the case of *E. ludwigii*. All fermentations in shake flasks were supplemented with the nutrient composition described in the preparation of pre-culture media.

The fruit extract, the vegetable hydrolysate, the commercial sugars and fermentation nutrients were sterilised separately at 121$^oC$ for 15 min. The inoculum used was 10% (v/v) and the working volume used in 500 mL Erlenmeyer flasks was 100 mL. All flasks were incubated at 30 $^oC$ in an orbital shaker with an agitation rate of 180 rpm. The pH was adjusted

to 7 at the beginning of fermentation and was not controlled during fermentation. The pH at the end of shake flask fermentations was around 5.8 – 6. The initial pH value used in shake flask cultures was higher than the one used in bioreactor cultures in order to allow enough time for the microbial strain to grow and consume the provided carbon source before the pH value became inhibitory to the microorganism.

All shake flask fermentations were carried out in triplicates and the results presented are the mean values of three biological replicates.

### 2.5 Bioreactor fermentations

Fed-batch bioreactor fermentations were carried out in a bench-top bioreactor (New Brunswick Scientific Co, USA) with 1 L total volume capacity and 600 mL working volume. Fruit extract or vegetable hydrolysates were used as carbon sources. Bioreactor cultures were supplemented with the nutrient composition described in the preparation of pre-culture media. The fruit extract, the vegetable hydrolysate and fermentation nutrients were sterilised separately at 121$^{o}$C for 15 min. The pH was controlled at 6.3 throughout fermentation using 5 M NaOH. Air supply was constant at 1 vvm. The dissolved oxygen was maintained at 5 % of saturation by agitation cascade in the range of 150 - 250 rpm. Both bioreactor cultures were carried out in duplicates.

The initial total sugar concentration in the fed-batch cultures carried out with either fruit extract or vegetable hydrolysate were 28.5 g/L and 15 g/L, respectively. The feeding solutions consisted of either concentrated fruit extract or concentrated vegetable hydrolysate at total sugar concentrations of 600 g/L and 300 g/L, respectively. Both feeding solutions were supplemented with 10 g/L yeast extract. The feeding rate in fed-batch cultures was controlled using a feed pump where the rate of sugar addition in the bioreactor was adjusted according to

the sugar consumption rate achieved by the microorganism as it was estimated after each sample. In this way, the flow rate of the feed pump was regulated at a constant value between samples in order to add continuously the feeding solution into the bioreactor. This approach minimises the inhibition that may be caused when the feeding medium is added in pulses into the bioreactor. However, a high quantity of feeding medium was added once at around 22 h only in the case of fed-batch fermentation carried out with fruit extract because the total sugar concentration was reduced overnight to almost zero. The total volume of feeding solutions of concentrated fruit extract and concentrated vegetable hydrolysate added in the fed-batch fermentations were 156 mL and 126 mL, respectively.

### 2.6 Analytical methods

Cellulose and hemicellulose contents were determined according to the analytical protocol reported by Sluiter et al. (2011). The ash content was determined by treating triplicate samples at 575 ◦C for 4 h. The moisture content in vegetables and fruits was determined by drying triplicate samples until constant weight via freeze drying.

Fermentation samples were taken at random times and the microbial cells were collected by centrifugation (9000 × g, 10 min, 4 °C). The sediment was treated twice by washing with distilled water, resuspension and centrifugation. The dry cell weight (DCW) was determined by drying the sediment from each fermentation sample at 50 °C until constant weight.

The sugars (glucose, xylose, galactose, mannose, arabinose, mannose, fructose and sucrose) and fermentation products (BDO, acetoin, lactic acid, succinic acid, acetic acid, ethanol) as well as furfural and 5-hydroxymethylfurfural (5-HMF) were quantified using a Shimadzu HPLC system equipped with a Shimadzu RI detector and an Aminex HPX–87H

column (7.8 × 300 mm, Bio-rad). A 10 mM $H_2SO_4$ solution was used as the mobile phase with 0.6 mL/min flow rate and 45°C column temperature. Sucrose was hydrolyzed by adding 100 µL of 10% (v/v) $H_2SO_4$ and 500 µL of sample in a test tube and boil at 100 °C for 30 min prior to HPLC analysis. The quantification of C5 and C6 sugars in vegetable hydrolysates was supported by a Shodex SP0810 column operated at 60 $^oC$ with 1 mL/min flow rate of HPLC grade water as the mobile phase.

## 3. **Results and Discussion**

### 3.1 Fruit and vegetable composition

Table 1 presents the cellulose and hemicellulose composition of the vegetables used in this study that was quantified based on the determination of structural monosaccharides. The cellulose content in the vegetables used in this study varied in the range of 11.9 – 26.2 g per 100 g dry solids, while the hemicellulose content varied within the range of 3.2 – 11.7 g per 100 dry solids. Vegetables contain various polysaccharides (e.g. starch, cellulose, hemicellulose, pectin) as well as sugars (e.g. glucose, fructose, sucrose) (Oke and Paliyath, 2006). Oke and Paliyath (2006) reported that carrots, green beans and lettuce contain a carbohydrate content of 79.8%, 69.7% and 53.8% on a dry basis. Rani and Kawatra (1994) reported that the composition of cellulose in carrots, cauliflower and cabbage was 15.93, 15 and 10.67 g per 100 g dry weight, while the composition of hemicellulose in the same vegetables was 1.8, 2.94 and 2.59 g per 100 g dry weight. The moisture content was around 90.1 – 94.9 %, whereas the ash content was lower than 1.8 g per 100 g dry solids in all vegetables. The free sugar content, mainly sucrose, glucose and fructose, was lower than 4.1 g per 100 g dry solids in all vegetables (Table 1).

### 3.2 Evaluation of vegetable hydrolysate production

Broccoli was selected as the substrate for the evaluation of sulphuric acid pretreatment conditions as it constituted around 80% of the vegetables used in this study. The aim was to evaluate the effect of dilute acid pre-treatment on the release of sugars mainly from hemicellulose that could be subsequently used for the production of BDO. The sugar recovery yield was calculated by dividing the sugars (i.e. xylose, galactose, arabinose) produced after dilute acid pre-treatment to the sum of the same sugars that were determined by the analytical protocol of Sluiter et al. (2011). There was no change in the glucose concentration before and after dilute acid treatment in all trials. Thus, cellulose was not hydrolysed. The hydrolysis of cellulose fraction was not attempted as this could be used for the production of ethanol. Glucose along with fructose were quantified before and after dilute acid hydrolysis and these are considered as free sugars because their concentration was not increased after hydrolysis.

Dilute acid pre-treatment led to the release of xylose, galactose and arabinose. Although the term hemicellulose hydrolysis has been used throughout this study, it should be stressed that a small fraction of these sugars could be also released from other vegetable components.

Two different initial solid concentrations of mixed vegetables (50 and 100 g/L, on a dry basis) were treated with 0.5, 1, 3 and 7% (v/v) $H_2SO_4$ solution for 30 min at 121 $^oC$ (Figure 1). Among the hydrolysis parameters that were tested, 3% and 7% of sulphuric acid concentration resulted in the highest hemicellulose hydrolysis yield. Specifically, when 3% (v/v) of sulphuric acid was used, hemicellulose hydrolysis yields (on a dry basis) of 65.8 % and 51.5 % were observed at 50 g/L and 100 g/L of initial solid concentration, respectively. When 7 % (v/v) of sulphuric acid concentration was used, the hemicellulose hydrolysis yields were 60.2 % and 47.8 % at 50 g/L and 100 g/L of initial solid concentration, respectively (Figure 1). The furfural concentration was 0.2 g/L and 0.8 g/L at 50 g/L and 100 g/L of initial solid concentration, respectively, when 3% (v/v) $H_2SO_4$ concentration was used. Higher

furfural concentrations (2 g/L and 2.5 g/L, respectively) were observed when 7% (v/v) sulphuric acid was used in 50 g/L and 100 g/L of initial solid concentrations. 5-HMF was not detected in any of the treatments. del Campo et al. (2006) evaluated the release of sugars (glucose and fructose) from various vegetables concluding that the maximum release of sugars from tomato (35.46%) and red pepper (50.2%) was achieved via mild hydrothermal pretreatment, while the maximum release of sugars from pulse food and artichoke (35.24%) required acid pretreatment.

Based on the results presented in Figure 1, the optimum conditions for the hydrolysis of hemicellulose fraction in broccoli were an initial solid concentration of 50 g/L (on a dry basis) treated at 121 $^{o}$C for 30 min with 3% (v/v) $H_2SO_4$. These conditions were subsequently tested in the hydrolysis of mixed vegetables resulting in approximately 65% of hemicellulose hydrolysis. This result complies with the hemicellulose hydrolysis yield achieved when only broccoli was used (Figure 1).

### 3.3 Shake flask fermentations using commercial sugars

Commercial glucose, xylose, galactose, mannose, arabinose, fructose and sucrose, representative sugars contained in vegetables and fruits, were selected as carbon sources in order to investigate the potential of BDO production by five bacterial strains. Table 2 presents the results obtained by each bacterial strain from all carbon sources used. The main product by all microorganisms was BDO, while acetoin, succinic acid and ethanol were also produced at lower concentrations. In the shake flask fermentations presented in Table 2, *E. ludwigii* FMCC 204 showed the highest productivities for the majority of the substrates tested. Specifically, the strain *E. ludwigii* achieved the highest BDO productivities in the case of glucose (1.01 g/L/h), mannose (0.86 g/L/h), xylose (0.68 g/L/h) and galactose (0.73 g/L/h). In

the case of fructose, *C. freundii* FMCC 207 resulted in higher productivity (0.88 g/L/h) than *E. ludwigii* (0.77 g/L/h), whereas in the case of arabinose and sucrose *E. aerogenes* FMCC 10 resulted in higher productivities than *E. ludwigii*. In the case of *E. aerogenes* FMCC 9, *Enterobacter* sp. FMCC 208 and *C. freundii* FMCC 207 the productivity range was 0.30 - 0.92 g/L/h, 0.38 - 0.88 g/L/h and 0.61 - 0.89 g/L/h, respectively. Saha and Bothast (1999) cultivated *E. cloacae* NRRL B-23289 using the same sugars at 50 g/L initial concentration in shake flask cultures and the highest productivity (0.56 g/L/h) was observed when fructose and arabinose were used, while the lowest productivity (around 0.3 g/L/h) was observed with glucose and xylose.

Sucrose resulted in the highest BDO to consumed carbon source conversion yield (0.4 g/g) by the majority of the strains used, except for *Enterobacter* sp. FMCC 208 (0.36 g/g). Conversion yields within the range of 0.34 - 0.4 g/g were predominantly observed by all strains in all substrates used with a few exceptions, such as *E. ludwigii* cultivation in arabinose (0.31 g/g), *E. aerogenes* FMCC 9 cultivation in arabinose (0.33 g/g) and *Enterobacter* sp. FMCC 208 cultivation in galactose (0.33 g/g).

Acetoin, succinic acid and ethanol were the main by-products in all shake flask fermentations (Figure 2, Table 2). Succinic acid and ethanol were produced by all microorganisms in all substrates at low concentrations (around 2 g/L). Succinic acid is produced through the oxidative branch of TCA cycle and its production is crucial for the regeneration of NADH and ATP.

Figure 2 presents the fermentation change of carbon source consumption as well as product and DCW formation in *E. ludwigii* cultures carried out in shake flasks using the commercial sugars presented in Table 2.

### 3.4 Shake flask fermentations using fruit extract and vegetable hydrolysate

Fermentations using the fruit extract were carried out by *E. ludwigii* with 25 g/L and 50 g/L initial total sugar concentration (Table 2 and Figure 3). Growth inhibition by initial sugar concentration was not observed. The final BDO concentration achieved from fruit extracts at 25 g/L and 50 g/L initial total sugar concentration was 10 g/L (Figure 3A) and 18.2 g/L (Figure 3B) with conversion yields of 0.4 g/g and 0.36 g/g, respectively. The productivity in both initial total sugar concentrations reached 1.25 g/L/h and 1.14 g/L/h, respectively. Figures 3C and 3D show that catabolite repression occurs between glucose and fructose consumption. Slow fructose consumption was observed when glucose was still available in the fermentation broth. Fructose was consumed faster when glucose was depleted from the fermentation medium. This phenomenon has been also observed by Jung et al. (2015) when *E. aerogenes* was cultivated in sugarcane molasses. By-product formation (Figures 3E and 3F) was at similar concentrations as the one observed when commercial sugars were used (Table 2).

The vegetable hydrolysate initially contained a furfural concentration of 0.2 g/L and a total sugar concentration of 6.08 g/L. The vegetable hydrolysate was concentrated to reach 15 g/L of initial total sugar concentration at the beginning of shake flask fermentation. The final BDO concentration achieved in shake flask cultures of *E. ludwigii* using the concentrated vegetable hydrolysate was 4.2 g/L with a conversion yield of 0.28 g/g and a productivity of 0.44 g/L/h (Table 2). The DCW was significantly lower (1.34 g/L) than the one obtained with the fruit extract (5 g/L) when 25 g/L of initial total sugar concentration was used.

Various renewable resources have been used for BDO production in shake flask fermentations. Perego et al. (2000) reported the cultivation of *Enterobacter aerogenes* NCIMB 10102 in starch hydrolysate, raw or decoloured molasses and whey and the highest yields and productivities were observed in starch hydrolysate and whey. Gao et al. (2010) reported the cultivation of *Paenibacillus polymyxa* ZJ-9 in shake flask cultures on raw inulin

extract from Jerusalem artichoke tubers leading to the production of 37.24 g/L R,R-2,3-BDO with a yield of 0.49 g/g. Wong et al. (2012) reported the cultivation of *Klebsiella* sp. on rice straw resulting in the production of 24.6 g/L BDO at a productivity of 2.41 g/L/h.

### 3.5 Bioreactor fed-batch fermentations using fruit and vegetable waste

Figure 4 presents the consumption of sugars as well as the production of DCW, BDO and by-products during fed-batch fermentation of *E. ludwigii* on the fruit extract. The initial total sugar concentration was 28.5 g/L and the feeding started at around 22 h, when the total sugar concentration was decreased to 10.7 g/L. Sugar consumption started after 4 h, while no lag phase was observed in the case of microbial growth. After 27 h, the glucose concentration in the fermentation broth was very low indicating that it was consumed rapidly during feeding of the concentrated fruit extract. Fructose accumulation was observed (Figure 4B) during feeding and the fructose concentrations was higher than 30 g/L until 100 h. Fructose consumption begun after the continuous feeding was stopped at around 100 h. As shown in shake flask fermentations (Figure 3), catabolite repression was also observed in the fed-batch bioreactor culture. The final BDO concentration was 50.1 g/L with a yield of 0.40 g/g and productivity of 0.41 g/L/h.

The main by-products produced during fed-batch fermentation were succinic acid, ethanol and lactic acid with final concentrations of 16.5 g/L, 5.8 g/L and 10.8 g/L. Succinic acid and ethanol act as final electron acceptors under oxygen limiting conditions. Although air was sparged continuously during fermentation, the dissolved oxygen concentration was always zero after 5 h indicating oxygen limiting conditions. Acetoin production was not observed in the fed-batch culture using fruit extract. Lactic acid production started at around

55 h when succinic acid production stopped. Lactic acid production was not observed in the case of the shake flask culture carried out with fruit extract (Figure 3).

Figure 5 presents the consumption of sugars as well as the production of DCW, BDO and by-products during fed-batch fermentation of *E. ludwigii* on the concentrated vegetable hydrolysate. The initial total sugar concentration was 15 g/L, while the furfural concentration was 0.5 g/L. Feeding was initiated at 8 h when the total sugar concentration was 4 g/L. The feeding solution contained 300 g/L of total sugars, 10 g/L yeast extract and 10 g/L furfural. The final BDO concentration was 17.6 g/L with a conversion yield of 0.32 g/g and a productivity of 0.39 g/L/h. The highest DCW was lower (4 g/L) than the DCW (8 g/L) produced in the fed-batch bioreactor culture using fruit extract (Figure 4A). Figure 5B shows that high concentrations of succinic acid (6.5 g/L), lactic acid (7.3 g/L) and ethanol (6.3 g/L) were produced during fermentation of vegetable hydrolysate. Acetoin was not detected during this fermentation. The fermentation stopped at 45 h probably due to the accumulation of inhibitory compounds, such as furfural and organic acids.

Cheng et al. (2010) utilised corncob hydrolysate in fed-batch cultures of *Klebsiella oxytoca* ACCC 10370 leading to the production of 35.7 g/L BDO with a yield of 0.5 g/g and a productivity of 0.59 g/L/h. Bialkowska et al. (2015) reported the cultivation of *Bacillus licheniformis* NCIMB 8059 in fed-batch cultures using enzymatic hydrolysate of depectinised apple pomace mixed with glucose (the feeding solution was glucose) leading to the production of 72.39 g/L BDO in 94 h when a 30 L bioreactor was employed. The bacterial strains *K. pneumoniae* has been used in fed-batch simultaneous saccharification and fermentation cultures employing Jerusalem artichoke powder and inulinase leading to the production of 91.63 g/L BDO in 40 h (Sun et al., 2009). Sikora et al. (2016) reported the production of 34.24 g/L of BDO at a productivity of 0.16 g/L/h and a yield of 0.35 g/g when *Bacillus amyloliquefaciens* was cultivated on apple pomace hydrolysate in fed-batch

bioreactor cultures (feeding was carried out with glucose), while when the same strain was cultivated on molasses the BDO production was 60.1 g/L (feeding was carried out with glucose) with a yield of 0.47 g/g and a productivity of 0.44 g/L/h. Priya et al. (2016) carried out fed-batch cultures in 150 L bioreactor with bacterial strain *Enterobacter cloacae* TERI BD 18 cultivated on glucose for the production of 85 g/L BDO with a productivity of 1.73 g/L/h and a yield of 0.48 g/g.

The utilisation of fruit and vegetable waste from open markets as feedstock for industrial chemical production should rely on the development of a biorefinery concept focusing on the extraction of value-added co-products (e.g. bioactive compounds, pectins) prior to the production of BDO via fermentation using the carbohydrate fraction. The biorefinery approach will create the profitability margin required for the reduction of the production cost of BDO. Logistics should be taken into consideration as a high number of open markets is only available in big cities. Koutinas et al. (2016) estimated that the minimum selling price of BDO is higher than 3 $/kg based on fermentation efficiencies reported in the literature using molasses or sucrose as feedstocks even at low market prices for these commodities. One of the biggest problems in BDO production is the development of a cost-competitive downstream separation process with low environmental impact.

## 4 Conclusion

Fruit and vegetables discarded as waste from open markets are rich in carbohydrates and can be used as fermentation media for the production of BDO. In this study, the bacterial strain *E. ludwigii* was initially selected as efficient BDO producer among five strains and it was subsequently evaluated for BDO production using fruit extracts and vegetable acid hydrolysates. Fed-batch cultures led to the production of 50 g/L of BDO from fruit waste and

17.6 g/L of BDO from vegetable acid hydrolysates. Further optimization of the fermentation process is required in order to achieve higher productivity, yield and final BDO concentration.

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**References**

Anonymous, 2017. FAOSTAT Production statistics. http://faostat3.fao.org/browse/Q/QC/E (visited on 10/04/2017)

Bialkowska, A.M., Gromek, E., Krysiak, J., Sikora, B., Kalinowska, H., Krzepkowska, M.J., Kubik, C., Lang, S., Schutt, F., Turkiewicz, M., 2015. Application of enzymatic apple pomace hydrolysate to production of 2,3-butanediol by alkaliphilic *Bacillus licheniformis* NCIMB 8059. J. Ind. Microbiol. Biotechnol. 42, 1609–1621.

Burniol-Figols, A., Cenian, K., Skiadas, I.V., Gavala, H.N. 2016. Integration of chlorogenic acid recovery and bioethanol production from spent coffee grounds. Biochem. Eng. J. 116, 54-64.

Celinska, E., Grajek, W., 2009. Biotechnological production of 2,3- butanediol - current state and prospects. Biotechnol. Adv. 27, 715-725.

Chandel, A.K., Antunes, F.A.F., de Arruda, P.V., Milessi, T.S.S., da Silva, S.S., Felipe, M.G.A., 2012. Dilute acid hydrolysis of agro-residues for the depolymerization of hemicellulose: state-of-the-art. Chapter 2. In: D-Xylitol – Fermentative production, applications and commercialization, da Silva, S.S., Chandel, A.K. (eds), Springer, pp. 39-62.

Cheng, K.K., Liu, Q., Zhang, J.A., Li, J.P., Xu, J.M., Wang, G.H., 2010. Improved 2,3-butanediol production from corncob acid hydrolysate by fed-batch fermentation using *Klebsiella oxytoca*. Process Biochem. 45, 613-616.

del Campo I., Alegria I., Zazpe M., Echeverria M., Echeverria I., 2006. Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production. Ind. Crop Prod. 24, 214–221.

Diaz, A.I., Laca, A., Laca, A., Diaz, M. 2017. Treatment of supermarket vegetable wastes to be used as alternative substrates in bioprocesses. Waste Manag. in press, doi.org/10.1016/j.wasman.2017.05.018

Doulgeraki, A. I., Paramithiotis, S., Nychas, G. J. E., 2011. Characterization of the *Enterobacteriaceae* community that developed during storage of minced beef under aerobic or modified atmosphere packaging conditions. Int. J. Food Microbiol 145: 77-83.

Drosinos, E. H., Mataragas, M., Xiraphi, N., Moschonas, G., 2005. Characterization of the microbial flora from a traditional Greek fermented sausage. Meat Sci. 69, 307-317.

Gao J, Xu H, Li QJ, Feng XH , Li S., 2010. Optimization of medium for one-step fermentation of inulin extract from Jerusalem artichoke tubers using *Paenibacillus polymyxa* ZJ-9 to produce R,R-2,3-butanediol. Bioresour Technol 101: 7087-7093.

Gustavsson J, Cederberg C, Sonesson U, Emanuelsson A. 2013. The methodology of the FAO study: ‘‘Global Food Losses and Food Waste – extent, causes and prevention” – FAO, SIK report No. 857.

Ji, X.J., Huang, H., Ouyang, P.K. 2011. Microbial 2,3-butanediol production: A state-of-the-art review. Biotechnol Adv. 29: 351–364.

Jung, M.Y., Jung, H.M., Lee, J., Oh, M.K. 2015. Alleviation of carbon catabolite repression in *Enterobacter aerogenes* for efficient utilization of sugarcane molasses for 2,3‑butanediol production. Biotechnol. Biofuels 8, 106-118.

Kim, D.-K., Park, J.M., Song, H., Chang, Y.K. 2016. Kinetic modeling of substrate and product inhibition for 2,3-butanediol production by *Klebsiella oxytoca*. Biochem. Eng. J. 114, 94–100.

Koutinas, A., Kookos, I.K. 2016. Editorial - Special issue on advances on biorefinery engineering and food supply chain waste valorization. Biochem. Eng. J. 116, 1–2.

Koutinas, A.A., Yepez, B., Kopsahelis, N., Freire, D.M.G., de Castro, A.M., Papanikolaou, S., Kookos, I.K. 2016. Techno-economic evaluation of a complete bioprocess for 2,3-butanediol production from renewable resources. Biores. Technol. 204, 55–64.

Lin, C.S.K., Koutinas, A.A., Stamatelatou, K., Mubofu, E.B., Matharu, A.S., Kopsahelis, N., Pfaltzgraff, L.A., Clark, J.H., Papanikolaou, S. Kwan, T.H., Luque, R. 2014. Current and future trends in food waste valorization for the production of chemicals, materials and fuels: a global perspective. Biofuels, Bioprod. Bioref. 8, 686–715.

Ventour, L. 2008. The food we waste. WRAP, Food waste report, Project code: RBC405-0010, ISBN: 1-84405-383-0

Mazumdar, S., Lee, J., Oh, M.K. 2013. Microbial production of 2,3-butanediol from seaweed hydrolysate using metabolically engineered *Escherichia coli*. Bioresour. Technol. 136, 329–336.

Metsoviti, M., Paramithiotis, S., Drosinos, E.H., Galiotou-Panayotou, M., Nychas, G.J.E., Zeng, A.P., Papanikolaou, S. 2012. Screening of bacterial strains capable of converting biodiesel-derived raw glycerol into 1,3-propanediol, 2,3-butanediol and ethanol. Eng. Life Sci. 12, 57-68.

Oke, M., Paliyath, G. 2006. Biochemistry of vegetable processing. Chapter 23. in: Food biochemistry and food processing, Hui, Y.H. (ed), Blackwell Publishing, pp. 537-554.

Palmqvist, E. Hahn-Hagerdal, B. 2000, Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. Bioresour. Technol. 74, 17-24.

Panda, S.K., Mishra, S.S., Kayitesi, E., Ray R.C. 2016. Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: Biotechnology and scopes. Env. Res. 146, 161–172.

Patsalou, M., Menikea, K.K., Makri, E., Vasquez, M.I., Drouza, C., Koutinas, M. 2017. Development of a citrus peel-based biorefinery strategy for the production of succinic acid. J. Cleaner Prod. 166, 706-716.

Perego, P., Converti, A., Del Borghi, A., Canepa, P. 2000. 2,3-Butanediol production by *Enterobacter aerogenes*: selection of the optimal conditions an application to food industry residues. Bioprocess Biosyst. Eng. 23, 613–620.

Priya, A., Dureja, P., Talukdar, P., Rathi, R., Lal, B., Sarma, P.M. 2016. Microbial production of 2,3-butanediol through a two-stage pH and agitation strategy in 150 l bioreactor. Biochem. Eng. J. 105, 159–167.

Rani, B., Kawatra, A. 1994. Fibre constituents of some foods. Plant Foods for Human Nutrition 45, 343-347.

Saha, B.C., Bothast, R.J. 1999. Production of 2,3-butanediol by newly isolated *Enterobacter cloacae*. Appl. Microbiol. Biotechnol. 52, 321-326.

Sikora, B., Kubik, C., Kalinowska, H., Gromek, E., Bialkowska, A., Krzepkowska, M.J., Schutt, F., Turkiewicz, M. 2016. Application of byproducts from food processing for production of 2,3-butanediol using *Bacillus amyloliquefaciens*. TUL 308. Prep. Biochem. Biotechnol. 46, 610–619.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2011. Determination of structural carbohydrates and lignin in biomass. Technical Report. NREL/TP-510-42618. www.nrel.gov/bioenergy/biomass-compositional-analysis.html.

Sun, L.H., Wang, X.D., Dai, J.Y., Xiu, Z.L. 2009. Microbial production of 2,3-butanediol from Jerusalem artichoke tubers by *Klebsiella pneumoniae*. Appl. Microbiol. Biotechnol. 82, 847–852.

Wong, C.L., Huang, C.C., Luc, W.B., Chen, W.M., Chang, J.S. 2012. Producing 2,3-butanediol from agricultural waste using an indigenous *Klebsiella* sp. Zmd30 strain. Biochem. Eng. J. 69, 32– 40.

Zeng, A.P., Sabra, W. 2011. Microbial production of diols as platform chemicals: Recent progresses. Current Opinion Biotechnol. 22, 749-757.

**Figures**

**Figure 1** Hemicellulose hydrolysis yield at 50 g/L (white bars) and 100 g/L (grey bars) initial solid concentration treated with four sulphuric acid concentrations (0.5%, 1%, 3% and 7% v/v) for 30 min at 121 $^{o}$C.

**Figure 2** Carbon source consumption and product generation using commercial substrate in shake flask fermentations carried out by *E. ludwigii* FMCC 204. Carbon source (■), BDO (●), dry cell weight (◊), acetoin (x), succinic acid (○), ethanol (▽).

**Figure 3** Total sugar consumption, DCW and BDO production (A and B), individual sugar consumption (C and E) and by-product generation (F and G) observed during shake flask fermentations carried out by *E. ludwigii* FMCC 204 when it was cultivated on fruit extract with initial sugar concentrations of 25 g/L (A, C, E) and 50 g/L (B, D, F). Total sugars (■), BDO (●), DCW (◊), glucose (▼), fructose (▲), sucrose (♦), acetoin (x), succinic acid (○), ethanol (▽).

**Figure 4** Total sugar consumption, DCW and BDO production (A), individual sugar consumption (B) and by-product generation (C) observed during fed-batch bioreactor fermentation carried out by *E. ludwigii* FMCC 204 when it was cultivated on fruit extract. Sucrose (♦), glucose (▼), fructose (▲), BDO (●), DCW (◊), succinic acid (○), lactic acid (□), ethanol (▽).

**Figure 5** Total sugar consumption, DCW and BDO production (A) and by-product generation (B) observed during fed-batch bioreactor fermentation carried out by *E. ludwigii* FMCC 204 when it was cultivated on vegetable acid hydrolysate. Total sugars (■), BDO (●), DCW (◊), succinic acid (○), lactic acid (□), ethanol (▽).

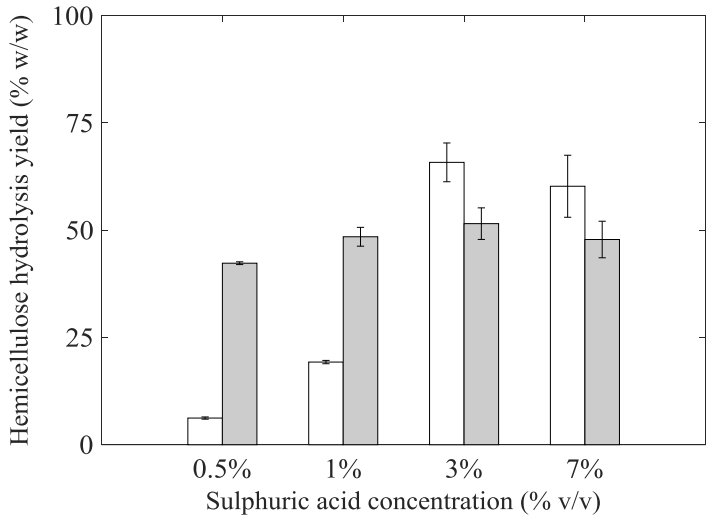


**Figure 1**

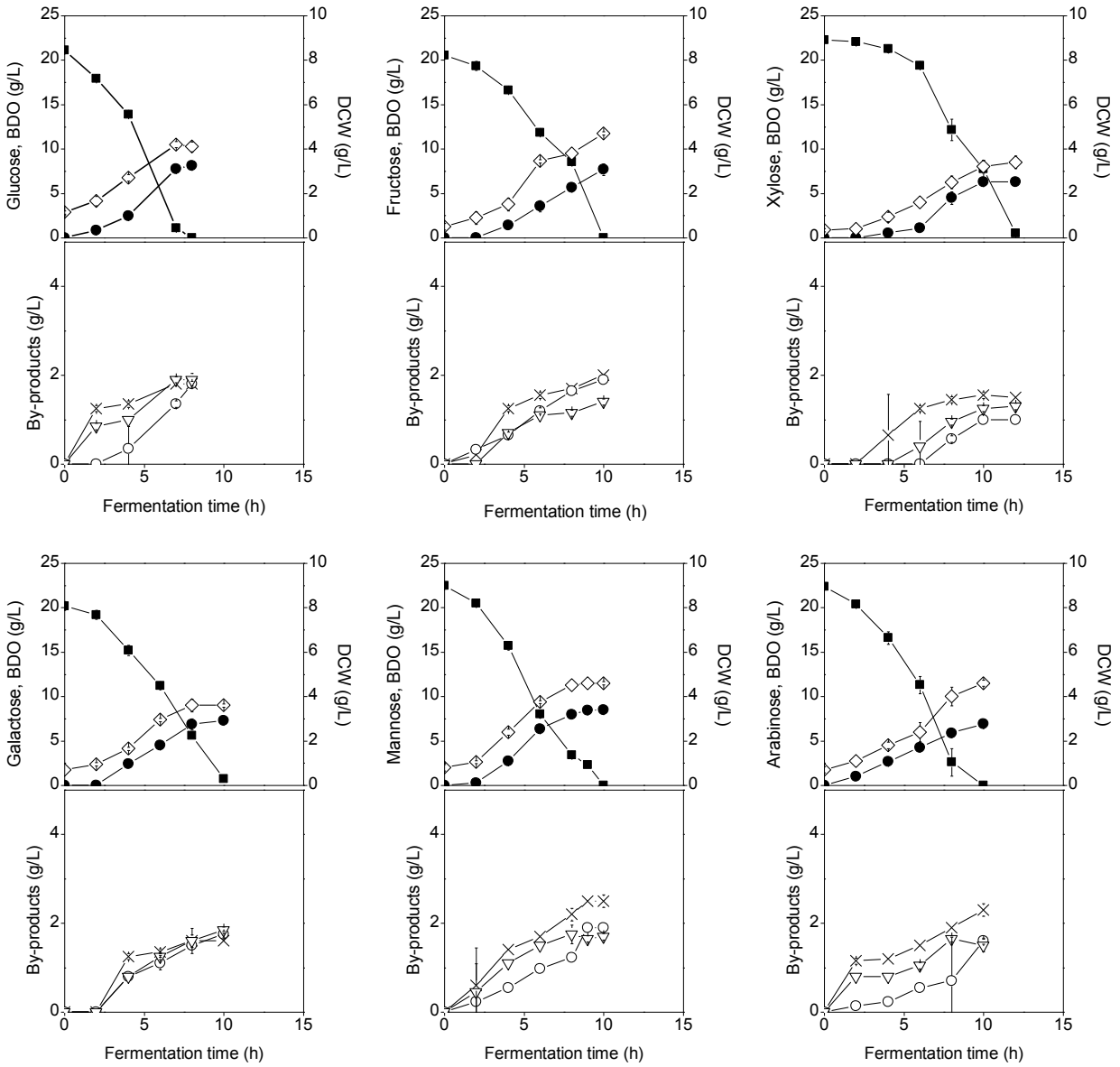


**Figure 2**

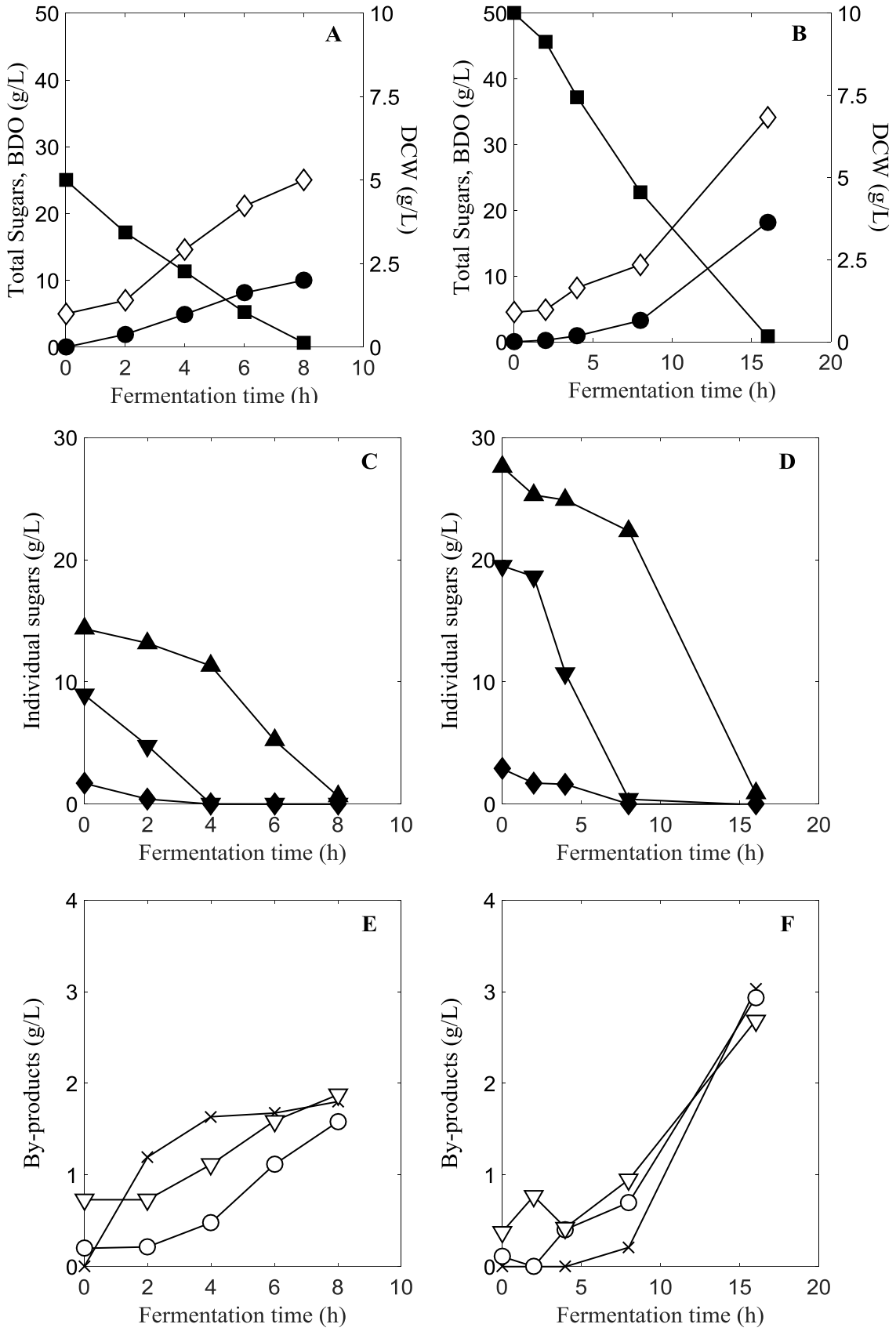


**Figure 3**

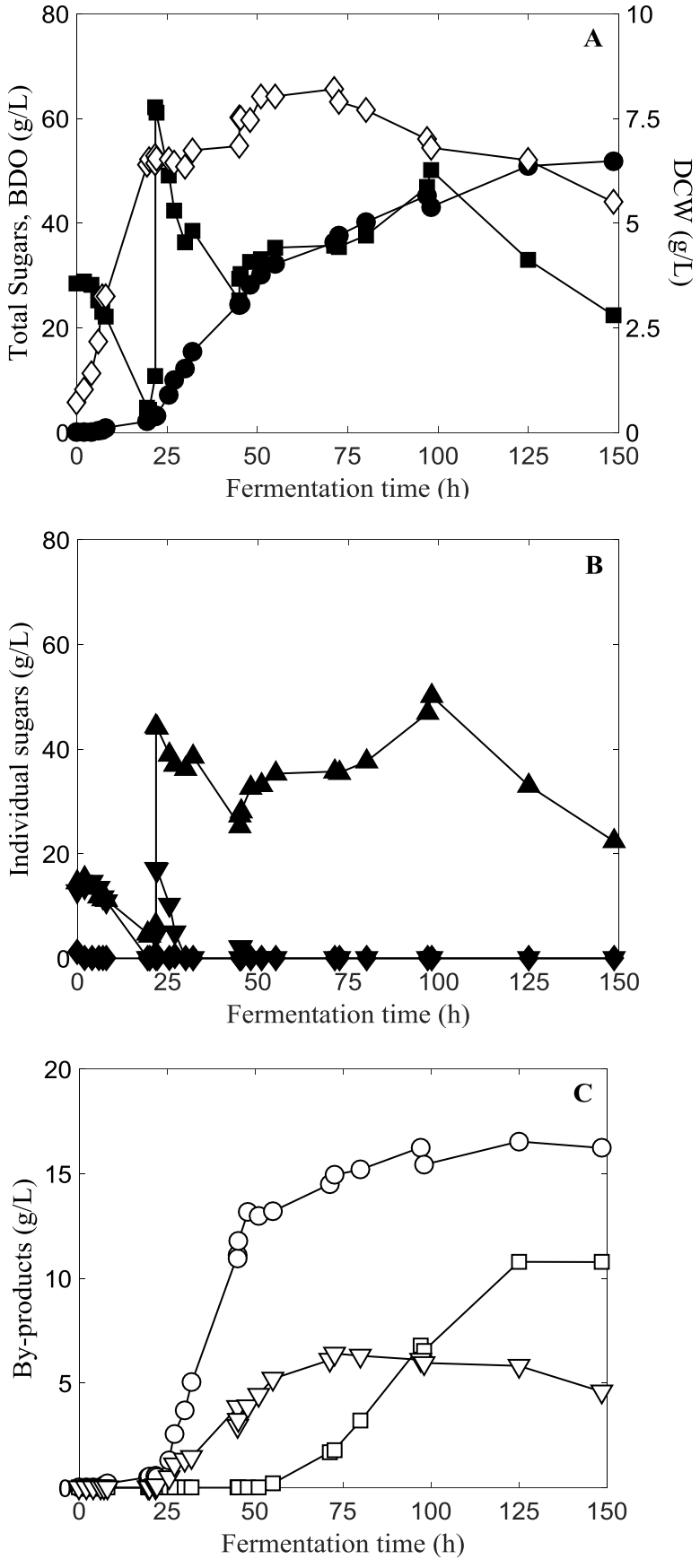


**Figure 4**

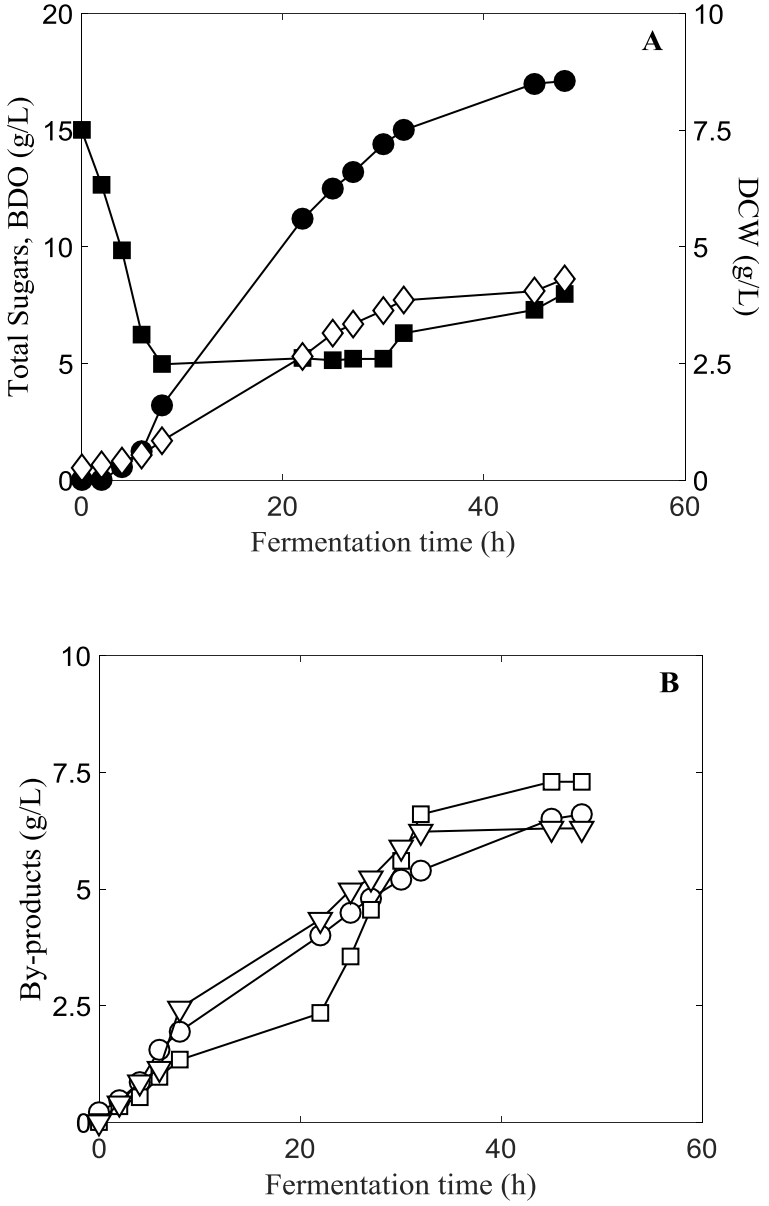


**Figure 5**

**Table 1** Composition of vegetables used in this study

| | Broccoli | Lettuce | Fresh beans | Corn salad / Tat soi salad | Cabbage | Carrots | Peppers (green and red) | Eggplant |
|---|---|---|---|---|---|---|---|---|
| Moisture (% wet basis) | 90.5 | 93.6 | 90.1 | 94.9 | 91.4 | 89.1 | 90.6 | 93.9 |
| Ash (g/100 g dry solids) | 0.75 | 1.2 | 1.6 | 0.5 | 1.4 | 0.62 | 1.8 | 0.26 |
| Free sugars (g/100 g dry solids) | 1.6 | 2.3 | 2.5 | 1.3 | 3.5 | 4.1 | 3.4 | 3.1 |
| Cellulose (g/100 g dry solids) | 15.0 | 12.8 | 26.2 | 20.9 | 25.6 | 11.9 | 16.2 | 17.3 |
| Hemicellulose (g/100 g dry solids) | 9.2 | 8.4 | 6.3 | 11.7 | 10.8 | 3.9 | 3.2 | 3.8 |
| Others (g/100 g dry solids) | 12.7 | 4.8 | 8.2 | 26.1 | 18.1 | 8.9 | 10.1 | 33.8 |

The results were obtained as the average of three replicates and standard deviation was lower than 0.5%.

**Table 2** Screening of five bacterial strains for BDO production in shake flasks with commercial substrates

| Bacterial strain | Substrate | % Meso – isomer | Total BDO (g/L) | Yield (g/g) | Productivity (g/L/h) | DCW (g/L) | Acetoin (g/L) | Succinic acid (g/L) | Ethanol (g/L) |
|---|---|---|---|---|---|---|---|---|---|
| *Enterobacter ludwigii* FMCC 204 | Glucose | 79 | 8.1 ± 0.07 | 0.38 ± 0.00 | 1.01 ± 0.01 | 4.1 ± 0.21 | 1.8 ± 0.07 | 1.8 ± 0 | 1.9 ± 0.14 |
| | Fructose | 76 | 7.7 ± 0.66 | 0.37 ± 0.03 | 0.77 ± 0.06 | 4.7 ± 0.07 | 2.0 ± 0.00 | 1.9 ± 0 | 1.4 ± 0.07 |
| | Mannose | 76 | 8.5 ± 0.23 | 0.38 ± 0.03 | 0.86 ± 0.03 | 4.6 ± 0.00 | 2.5 ± 0.00 | 1.9 ± 0 | 1.7 ± 0.07 |
| | Arabinose | 76 | 6.9 ± 0.01 | 0.31 ± 0.00 | 0.69 ± 0.00 | 4.6 ± 0.14 | 2.3 ± 0.14 | 1.6 ± 0.07 | 1.5 ± 0.14 |
| | Xylose | 77 | 6.3 ± 0.00 | 0.38 ± 0.00 | 0.68 ± 0.00 | 3.4 ± 0.28 | 1.5 ± 0.00 | 1.0 ± 0 | 1.3 ± 0.07 |
| | Galactose | 75 | 7.3 ± 0.63 | 0.37 ± 0.03 | 0.73 ± 0.08 | 3.6 ± 0.28 | 1.6 ± 0.28 | 1.5 ± 0 | 1.6 ± 0.14 |
| | Sucrose | 75 | 18.3 ± 1.20 | 0.40 ± 0.02 | 0.91 ± 0.06 | 5.7 ± 0.57 | 2.5 ± 0.10 | 1.8 ± 0.07 | 2.0 ± 0.07 |
| | Vegetable hydrolysate ($S_0$ 15 g/L)[1] | 76 | 4.2 ± 0.14 | 0.28 ± 0.2 | 0.44 ± 0.18 | 1.34 ± 0.07 | 0.99 ± 0.01 | 1.9 ± 0 | 2.4 ± 0.14 |
| | Fruit extract ($S_0$ 25 g/L) | 78 | 10 ± 0.2 | 0.40 ± 0.12 | 1.25 ± 0.2 | 5 ± 0.07 | 1.8 ± 0.04 | 1.6 ± 0.03 | 1.9 ± 0.02 |
| | Fruit extract ($S_0$ 50 g/L) | 77 | 18.2 ± 0.43 | 0.36 ± 0.33 | 1.14 ± 0.43 | 6.8 ± 0.03 | 3.0 ± 0.07 | 2.9 ± 0.04 | 2.7 ± 0.09 |
| *Enterobacter aerogenes* FMCC 9 | Glucose | 81 | 6.9 ± 0.14 | 0.37 ± 0.03 | 0.58 ± 0.01 | 2.8 ± 0.35 | 1.9 ± 0.07 | 0.8 ± 0.21 | 1.0 ± 0 |
| | Fructose | 72 | 7.7 ± 0.21 | 0.34 ± 0.01 | 0.38 ± 0.01 | 3.5 ± 0.07 | 1.5 ± 0.14 | 2.3 ± 0 | 1.5 ± 0.07 |
| | Mannose | 73 | 7.3 ± 0.42 | 0.37 ± 0.01 | 0.61 ± 0.04 | 3.4 ± 0.21 | 1.8 ± 0.00 | 1.5 ± 0 | 1.9 ± 0.07 |
| | Arabinose | 72 | 7.3 ± 0.14 | 0.33 ± 0.03 | 0.61 ± 0.01 | 2.8 ± 0.07 | 1.7 ± 0.07 | 1.0 ± 0.07 | 1.7 ± 0.14 |
| | Xylose | 73 | 7.1 ± 0.11 | 0.36 ± 0.01 | 0.30 ± 0.00 | 3.3 ± 0.07 | 2.0 ± 0.00 | 1.7 ± 0.07 | 1.5 ± 0 |
| | Galactose | 63 | 6.5 ± 0.07 | 0.34 ± 0.00 | 0.59 ± 0.01 | 2.6 ± 0.07 | 1.4 ± 0.00 | 1.1 ± 0.07 | 1.3 ± 0.07 |
| | Sucrose | 72 | 18.4 ± 2.12 | 0.40 ± 0.06 | 0.92 ± 0.11 | 5.7 ± 0.42 | 2.8 ± 0.25 | 1.5 ± 0.24 | 2.4± 0.14 |
| *Enterobacter aerogenes* | Glucose | 77 | 7.9 ± 0.64 | 0.37 ± 0.04 | 0.79 ± 0.06 | 2.4 ± 0.07 | 1.7 ± 0.42 | 1.2 ± 0.14 | 1.4 ± 0.42 |
| | Fructose | 69 | 7.7 ± 0.14 | 0.37 ± 0.03 | 0.43 ± 0.01 | 3.7 ± 0.14 | 1.4 ± 0.07 | 2.7 ± 0.07 | 1.7 ± 0.28 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| FMCC 10 | Mannose | 79 | 8.0 ± 0.02 | 0.36 ± 0.00 | 0.80 ± 0.00 | 3.5 ± 0.00 | 1.7 ± 0.28 | 2.2 ± 0.07 | 2.1 ± 0.07 |
| | Arabinose | 77 | 7.4 ± 0.08 | 0.34 ± 0.02 | 0.74 ± 0.08 | 2.9 ± 0.21 | 1.9 ± 0.14 | 1.5 ± 0.07 | 2.4 ± 0.07 |
| | Xylose | 68 | 7.9 ± 0.14 | 0.36 ± 0.04 | 0.40 ± 0.01 | 3.3 ± 0.14 | 1.5 ± 0.07 | 0.8 ± 0.14 | 1.0 ± 0 |
| | Galactose | 73 | 6.9 ± 0.28 | 0.34 ± 0.04 | 0.69 ± 0.03 | 2.9 ± 0.14 | 1.5 ± 0.00 | 1.8 ± 0.14 | 1.7 ± 0 |
| | Sucrose | 70 | 20.2 ± .07 | 0.40 ± 0.06 | 1.01 ± 0.00 | 6.0 ± 0.11 | 2.1 ± 0.12 | 1.5 ± 0.17 | 1.3 ± 0.07 |
| *Enterobacter* sp. FMCC 208 | Glucose | 83 | 7.5 ± 0.28 | 0.38 ± 0.00 | 0.63 ± 0.02 | 2.7 ± 0.28 | 2.3 ± 0.35 | 0.7 ± 0.07 | 1.0 ± 0.07 |
| | Fructose | 71 | 7.8 ± 0.14 | 0.38 ± 0.03 | 0.39 ± 0.01 | 3.6 ± 0.07 | 1.7 ± 0.07 | 2.6 ± 0.14 | 2.0 ± 0.07 |
| | Mannose | 76 | 7.6 ± 0.21 | 0.34 ± 0.03 | 0.64 ± 0.02 | 3.7 ± 0.42 | 1.9 ± 0.07 | 1.6 ± 0.14 | 1.7 ± 0.21 |
| | Arabinose | 71 | 6.9 ± 0.64 | 0.38 ± 0.04 | 0.38 ± 0.04 | 3.7 ± 0.07 | 1.5 ± 0.07 | 1.2 ± 0.07 | 1.3 ± 0.14 |
| | Xylose | 71 | 7.9 ± 0.21 | 0.37 ± 0.01 | 0.39 ± 0.01 | 3.9 ± 0.14 | 1.4 ± 0.00 | 1.1 ± 0.07 | 1.0 ± 0 |
| | Galactose | 70 | 6.3 ± 0.49 | 0.33 ± 0.04 | 0.63 ± 0.05 | 2.7 ± 0.00 | 1.4 ± 0.00 | 1.7 ± 0.07 | 1.7 ± 0.28 |
| | Sucrose | 68 | 17.6 ± 1.56 | 0.36 ± 0.02 | 0.88 ± 0.08 | 5.8 ± 0.42 | 2.0 ± 0.09 | 1.5 ± 0.16 | 1.9 ± 0.23 |
| *Citrobacter freundii* FMCC 207 | Glucose | 69 | 7.0 ± 0.00 | 0.36 ± 0.00 | 0.87 ± 0.00 | 4.3 ± 0.21 | 3.1 ± 0.07 | 1.8 ± 0 | 2.0 ± 0.21 |
| | Fructose | 66 | 7.7 ± 0.07 | 0.35 ± 0.01 | 0.88 ± 0.01 | 3.9 ± 0.07 | 2.9 ± 0.14 | 1.8 ± 0.07 | 2.3 ± 0.28 |
| | Mannose | 73 | 8.3 ± 0.34 | 0.36 ± 0.01 | 0.83 ± 0.08 | 4.8 ± 0.07 | 1.9 ± 0.00 | 1.8 ± 0.84 | 2.3 ± 0 |
| | Arabinose | 72 | 7.6 ± 0.14 | 0.36 ± 0.02 | 0.76 ± 0.01 | 4.0 ± 0.07 | 1.9 ± 0.14 | 1.4 ± 0.14 | 2.3 ± 0 |
| | Xylose | 64 | 8.6 ± 0.28 | 0.36 ± 0.03 | 0.61 ± 0.02 | 3.7 ± 0.07 | 1.4 ± 0.00 | 1.2 ± 0.14 | 1.6 ± 0.07 |
| | Galactose | 71 | 6.8 ± 0.11 | 0.35 ± 0.00 | 0.68 ± 0.01 | 4.2 ± 0.07 | 1.7 ± 0.07 | 1.2 ± 0.14 | 1.9± 0.21 |
| | Sucrose | 67 | 19.6 ± 0.21 | 0.40 ± 0.01 | 0.89 ± 0.01 | 5.4 ± 0.71 | 1.7 ± 0.25 | 2.6± 0.24 | 2.0± 0.12 |

[1] $S_0$: Initial sugar concentration