Turkish Journal of Agriculture - Food Science and Technology, 10(12): 2300-2306, 2022
DOI: https://doi.org/10.24925/turjaf.v10i12.2300-2306.5091

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Effect of Carbon Sources on Glutamate Production from *Corynebacterium glutamicum* 2262

Abdenacer Mouffok[1,a*], Djedjiga Bellouche[1,b], Nabil Nancib[1,c], Joseph Boudrant[2,d]

[1]*Laboratory of Applied Microbiology, Ferhat Abbas University, Setif-1, Setif 19000, Algeria*
[2]*Laboratory Reactions and Chemical Engineering (LRGP), University of Nancy, Vandoeuvre-les Nancy 54505, France*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 03/02/2022
Accepted : 06/09/2022

Keywords:
Date syrup
Glutamate
Corynebacterium glutamicum 2262
Batch culture
Carbon sources.

ABSTRACT

A comparative study between natural and synthetic environments is carried out by realizing six fermentation experiments in batch culture. The objective of this study is to consider the effect of carbon sources on growth and the production of glutamic acid by *Corynebacterium glutamicum* 2262. The difference between the culture media lies in the carbon source. Two natural environments of date juice, one of which is treated with invertase to hydrolyse sucrose with a concentration of total sugars of 88 g/L. Four synthetic media with a concentration of 34 g/L sugars: medium containing mixed sugars (glucose + fructose + sucrose) with rates proportional to that of date juice, the other three media are composed of a single sugar (either glucose, fructose, or sucrose). The results showed that *Corynebacterium glutamicum* is able to use the three sugars whether they are single or mixed, although the best results of glutamate production (8.41 g/L) are obtained on the mixture of three sugars, which explains the interest and valorisation of date waste. On the other hand, the date juice-based media are shown to have a glutamate concentration of 7.98 g/L during the hydrolysis of sucrose of date juice.

[a] *mouffok_adel@univ-setif.dz* *https://orcid.org/0000-0001-7515-4651*
[b] *djedjigabellouche@gmail.com* *https://orcid.org/0000-0002-5824-9920*
[c] *nancibnabil@yahoo.fr* *https://orcid.org/0000-0003-4506-4625*
[d] *joseph.boudrant@univ-lorraine.fr* *https://orcid.org/0000-0001-5392-351X*

This work is licensed under Creative Commons Attribution 4.0 International License

Introduction

The depletion of oil resources, the uneven distribution of fossil fuels in the world and the alarming emissions of greenhouse gases has caused a revival in the valorisation of bio-transformations (microbial or enzymatic) from renewable resources.

Amino acids are commercially important biological components. The largest market among amino acids is that of glutamate. It is used as an additive in food, feed supplement, infusion compound, therapeutic agents and precursors for peptide synthesis or in chemical product-based agriculture. It is mainly used as a flavour enhancer and has become an important product for industrial exploitation of microbiology (Tavakkoli et al., 2009). The global annual production of monosodium glutamate has reached more than 2.75 million tonnes in recent years, of which about 80% (2.2 million tonnes) is produced in China (Liming et al., 2018 ; Dong et al., 2020).

Since its discovery, *Corynebacterium glutamicum* is industrially considered as a very important microorganism. Indeed, it has many intrinsic properties that make it a potential cell factory to produce primary metabolites (Hideaki and Masayuki, 2013).

Different raw materials such as wheat hydrolysate, beet and cane molasses (Shaik et al., 2011;

Ganguly and Banik, 2012) and date juice (MouffoK et al., 2012) are used industrially to produce glutamic acid. Three culture processes do the production of glutamate by fermentation: batch, fed-batch and continuous (Mouffok et al., 2021). Dates are rich in certain nutrients and provide a good source of fast energy, due to their high carbohydrate content (El Arem et al., 2011).

Annual date production in Algeria is estimated to be 789,350 50 tonnes (Benyahia-Krid et al., 2021). Dates have already been tested to be exploited as a fermentation feedstock to produce various metabolites, such as citric acid, oxytetracycline, ethanol, feed production, etc.

In this work, we sought to determine the effect of carbon sources on the production of glutamate by *Corynebacterium glutamicum* 2262 using the batch culture method. To do so we have used natural media of dates juice, synthetic media containing a single source of carbon, and a simulated medium to that of date juice containing the glucose, fructose and sucrose. This will allow us to know the behavior of the strain on the sugars of the date juice.

The enzymatic hydrolysis of sucrose in date juice will also be tested in order to improve the performance of glutamic fermentation.

Materials and Methods

Microorganism and preparation of inoculum

The strain used throughout this study was thermo-inducible *Corynebacterium glutamicum* 2262 (glutamate production induced by a temperature shift) (Delaunay et al., 1999) provided by Amylum-Orsan (France). The inoculum was grown in a shake-flask culture at 33°C in Medium of ***C****orynebacterium* ***g****lutamicum* enriched with **C**itrate **M**odified **M**edium (CGCMM) (Von der Osten et al., 1989) with the following composition: glucose, 34 g/L; urea, 4 g/L; thiamine, 20 mg/L; biotin, 2 mg/L; $CaCl_2$, 84 mg/L; $MgSO_4.7H_2O$, 400 mg/L; $FeSO_4.7H_2O$, 40 mg/L; $FeCl_3$, 4 mg/L; $ZnSO_4.7H_2O$, 1 mg/L; $CuCl_2.2H_2O$, 0.4 mg/L; $MnSO_4.H_2O$, 1 g/L; $(NH_4)_2SO_4$, 8 g/L.

Extraction of the date juice

The substrate used was obtained from date rejects. The dates were thoroughly cleaned manually to remove dust and foreign materials. The method used for sugar extraction from these dates was adapted from (Nancib et al., 1999). The amount of water to be added is two litters per kilogram of date pulp. The mixture is heated at 90 °C for two hours in a water bath with continuous stirring. The obtained juice extract is centrifuged at 10,000 x g for 10 minutes to separate the cellulosic debris. The collected supernatant is used essentially as the carbon source in the fermentation medium after being diluted to the desired concentration of total sugars. The total sugar content (glucose, fructose and sucrose) of the collected supernatant was determined.

The date juice sugars were used as carbon source with the nutrient supplement of nitrogen sources: ammonium sulphate (Sigma), and vitamins (biotin and thiamine [Sigma], minerals, and glycine betaine [Sigma]). The pH of the medium is adjusted (7.6) before proceeding with the fermentation.

Fermentation medium

The used fermentation medium is based on date juice sugars as a source of carbon 88 g/L. The medium was sterilized at 121°C for 20 min. This medium groups together the requirements of the strain in carbon and nitrogen sources, mineral and vitamin. Its composition will vary depending on the experiments carried out: carbon sources (glucose, fructose, sucrose single or mixed and juice date) supplemented with ammonium sulfate, 8 g/L; $MgSO_4.7H_2O$, 0.6 g/L; biotin, 272 µg/L; thiamine, 0.01 g/L; glycine betaine, 10 g/L and minerals ($CaCl_2$, 0.01 g/L; KH_2PO_4, 2.5 g/L; K_2HPO_4, 2.5 g/L, $MgSO_4$, 0.6 g/L; $MnSO_4$, 2.5 mg/L) (Mouffok et al., 2012).

Culture in Erlenmeyer flasks

The optimization study experiments were carried out in 500 mL Erlenmeyer flasks, containing 100 mL cultivation medium. The air enters and leaves through the sterilized cotton plugging the flasks. The cultivation medium occupies $1/5^{th}$ of the flask volume where a large dead volume ($4/5^{th}$ of the volume of the flask) is occupied by air. In addition, the cultivation medium was inoculated with 11.5% (V/V) of seed inoculums and then the flasks were incubated on rotary shaker at 330 rpm. The pH was adjusted to 7.6. The increase in the culture temperature (from 33 to 39°C) was performed when the exponential phase was attained. The flasks have the advantage of being able to conduct several of fermentations, at the same time, a lower cost, and with easier implementation.

Analytical methods

Cell biomass concentration was estimated by optical density measurement at 570 nm and calibrated to the cell dry weight. Glutamate and sugars (glucose, fructose and sucrose) were measured using enzymatic kit from Roche Biopharm (Darmstadt, Germany).

Results and discussion

The objective of this study was to show the fermentation performance of *Corynebacterium glutamicum* 2262 on date juice with a medium (synthetic medium: CGCMM) optimized in a previous work (Mouffok et al., 2012) with the three sugars mixture (glucose, fructose and sucrose) and the same proportions as it was for the date juice; to show the effect of the presence of only one single sugar (glucose, fructose or sucrose) in the CGCMM on the kinetics of *Corynebacterium glutamicum* 2262; and finally to study the hydrolysis of sucrose of date juice in order to improve the performance of glutamic fermentation.

Kinetics of Corynebacterium glutamicum 2262 on date juice

Fermentation in batch culture was carried out on date juice containing an initial concentration of total sugars of 88 g/l. This concentration corresponds to the following proportions: 27% glucose equivalent to 23.76 g/L, 23% fructose equivalent to 20.24 g/L and 50% sucrose equivalent to 44 g/L. The inoculum rate 11.5%, 14 h age of inoculum, 370 rpm stirring, pH 7.6, temperature 33°C and then a thermal shock at 39°C. The batch culture conditions were optimized by Mouffok et al. (2012).

The kinetics of growth, sugar consumption, and glutamic acid production during batch cultures on date juice medium as a carbon source are shown in Figure 1. Several kinetic parameters have been calculated (Table 1). The obtained results showed that the three sugars are consumed simultaneously with a specific rate of substrate consumption 1.86 g/g.h and a percentage substrate consumption of 92.53%. The maximum concentration of glutamate was 7.55 g/L, the volumetric productivity of glutamic acid was 0.71 g/L.h. This was confirmed by several authors who cited the ability of *Corynebacterium glutamicum* to use different carbon sources (Dominguez and Lindley, 1996). The consumption rate of each sugar of various mixtures still depended on sugar transport capacities (Dominguez and Lindley, 1996). Because the date juice contains a mixture of sugars (glucose, fructose and sucrose), it would be interesting to study the kinetics of glutamate production and consumption of different sugars by *Corynebacterium glutamicum* under different conditions: natural and synthetic media. This can give an idea on the behaviour of the strain.

Table 1. Kinetic parameters of *C. glutamicum* 2262 on date juice in batch culture.

X_{max} (g/L)	Glutamate max (g/L)	μ_{max} (1/h)	q_s (g/g.h)	Substrate consumption rate (%)	δ (g/L.h)
5.84	7.55	0.26	1.86	92.53	0.71

X: biomass; μ_{max}: maximum specific growth rate; q_s: specific rate of substrate consumption, (%); (δ): volumetric productivity of glutamic acid production.

Table 2. Kinetic parameters of *C. glutamicum* 2262 mixed sugars (glucose + fructose + sucrose) in batch culture.

X max (g/L)	Glutamate max (g/L)	μ_{max} (1/h)	q_s (g/g.h)	Substrate consumption rate (%)	δ (g/L.h)
5.18	8.41	0.24	3.49	92.15	0.68

X: biomass; μ_{max}: maximum specific growth rate; q_s: specific rate of substrate consumption. (%); (δ): volumetric productivity of glutamic acid production.

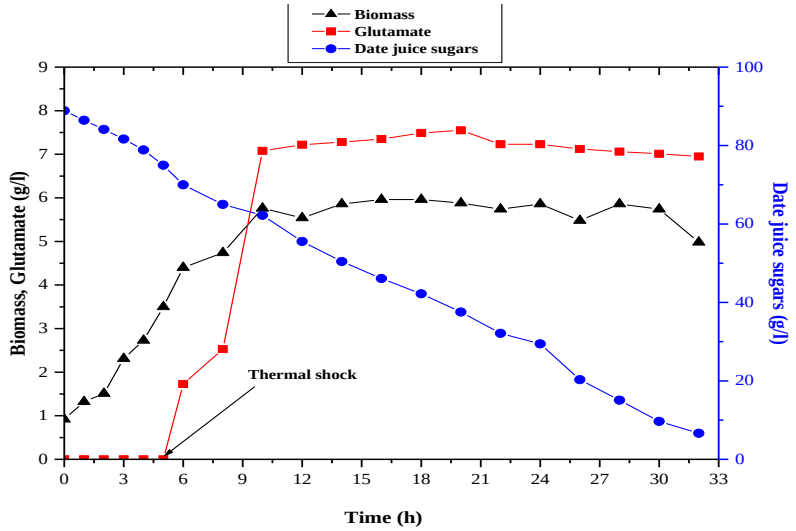

Figure 1. Kinetics of *C. glutamicum* 2262 on date juice in discontinuous culture

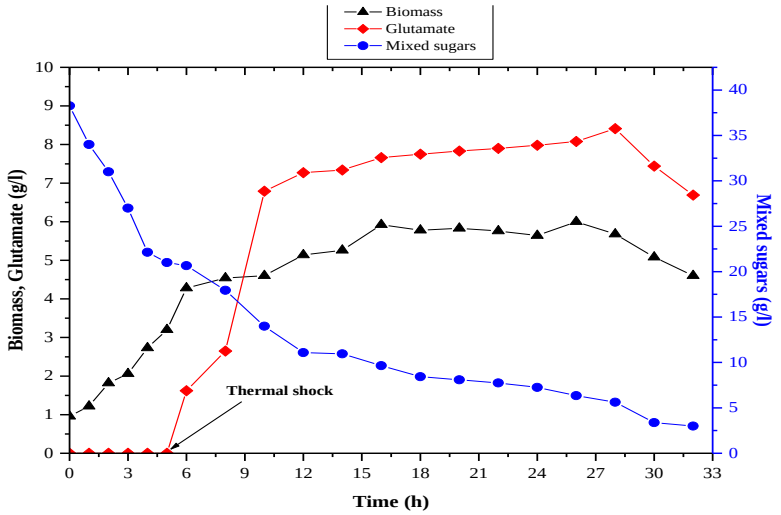

Figure 2. Kinetics of *Corynebacterium glutamicum* 2262 on mixed sugars (glucose + fructose + sucrose) in batch culture.

Kinetics of Corynebacterium glutamicum 2262 on the mixture of the three sugars

The kinetics of growth, sugar consumption and glutamic acid production during batch cultures on CGCMM containing the three sugars (glucose + fructose + sucrose) as a carbon source are shown in Figure 2.

After analysing the obtained results on date juice, it was necessary to study the effect of the kinetics of *Corynebacterium glutamicum* on the mixture of the three sugars with a total sugar concentration of 34 g/L, and with the same proportions of the sugars of date juice: 27% glucose equivalent to 9.18 g/L, 23% fructose equivalent to 7.82 g/L and 50% sucrose equivalent to 17 g/L.

As for the other cultures, the induction of the production of glutamate is made 5 h after fermentation (during the exponential phase) by raising the temperature from 33 to 39°C. Several parameters are calculated to allow comparing between the previous natural environment and this synthetic medium. The obtained results are summarized in Table 2.

The maximum concentration of biomass (5.18 g/L) and the growth rate (0.24 1/h) are not very different from those obtained on the natural environment based on date juice (5.84 g/L and 0.26 1/h), respectively. The specific rate of substrate consumption of 3.49 g/g.h for the synthetic medium is higher than that obtained on the natural medium (1.86 g/g.h). On the other hand, the maximum concentration of glutamate on date juice (7.55 g/L) is lower than that obtained on the mixture of the three sugars (8.81 g/L). The volumetric productivity of glutamic acid production was almost the same on the natural and synthetic medium which were 0.71 and 0.68 g/L.h, respectively. All sugars were consumed with a consumption rate of 92.15% for the mixture of the three sugars and 92.53% for date juice sugars. This result in agreement with the result obtained by Dominguez et al. (1997). Indeed, when *Corynebacterium glutamicum* or *Enterococcus faecalis* grow on a mixture of sugars, the consumption of these sugars is simultaneous, and the results are better than those obtained on sugars alone.

It has been suggested (Dominguez et al., 1997) that the major limiting factor of *Corynebacterium glutamicum* amino acid production is the potential of these bacteria to transport the sugars. The use of sugar mixtures can help to overcome this limitation. Péquignot et al. (1997) noted that the proportions of sugars (glucose and fructose) in a mixed medium have remarkable effects on glutamate production.

Corynebacterium glutamicum is able to use many carbonaceous substrates including sugars, organic acids, nucleic acids, alcohols and aromatic compounds (Teramoto et al., 2011). Sugars, particularly glucose, sucrose and fructose are the preferred carbon sources in industrial biotechnology processes. These sugars can enter the cell by two routes: the phosphotransferase system (PTS) and the permease system (Figure 3). The PTS system plays a major role in the entry of sugars into *Corynebacterium glutamicum* cells and catalyzes the phosphorylation of these sugars (either glucose-6-phosphate or fructose-6-phosphate) using phosphoenolpyruvate (PEP) and generating pyruvate (Dominguez and Lindley, 1996). Let us add that intracellular fructose, which is formed during growth from sucrose, is first eliminated by an unknown export system, then is captured and phosphorylated into fructose-1-phosphate by a PTS (ptsF) (Dominguez and Lindley, 1996).

Kinetics of Corynebacterium glutamicum 2262 on single sugars

Figures 4(a, b, c) show the evolution of sugar concentrations in a medium containing either glucose, fructose or sucrose, biomass and glutamic acid production over the time. The obtained results show that the three sugars are favourable for the growth of the strain and the production of glutamic acid. From the results described above, several kinetic parameters were calculated to compare the fermentation performance on each sugar: growth, sugar consumption rate, maximum growth rate and the volumetric productivity of glutamic acid production. The obtained kinetic parameters are summarized in Table 3.

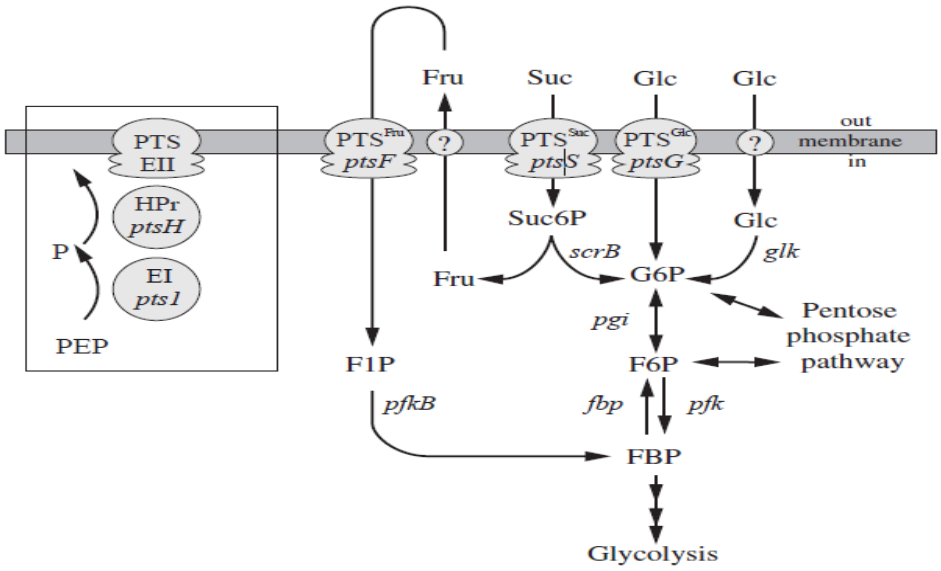

Figure 3. Sugar transport systems in *Corynebacterium glutamicum* (Eggeling and Bott, 2005)
PTSG1c: PTS glucose; PTSFru: PTS fructose; PTSsuc: PTS sucrose; unidentified transport system. Fru: fructose; Suc: sucrose; Glc: glucose; G6P: glucose-6-phosphate; F6P: fructose-6-phosphate; FBP: fructose-1,6-diphosphate; Suc6P: sucrose-6-phosphate

Table 3. Kinetic parameters of *C. glutamicum* 2262 on single sugars in batch cultures.

Carbon Source	X max (g/L)	Glutamate max (g/L)	μ_{max} (1/h)	q_s (g/g.h)	Substrate consumption rate (%)	δ (g/L.h)
Glucose	6.76	6.36	0.29	1.92	92.53	0.63
Fructose	7.06	7.08	0.18	2.99	87.43	0.65
Sucrose	9.52	6.58	0.29	2.84	93.86	0.66

X: biomass; μ_{max}: maximum specific growth rate; q_s: specific rate of substrate consumption. (%); (δ): volumetric productivity of glutamic acid production.

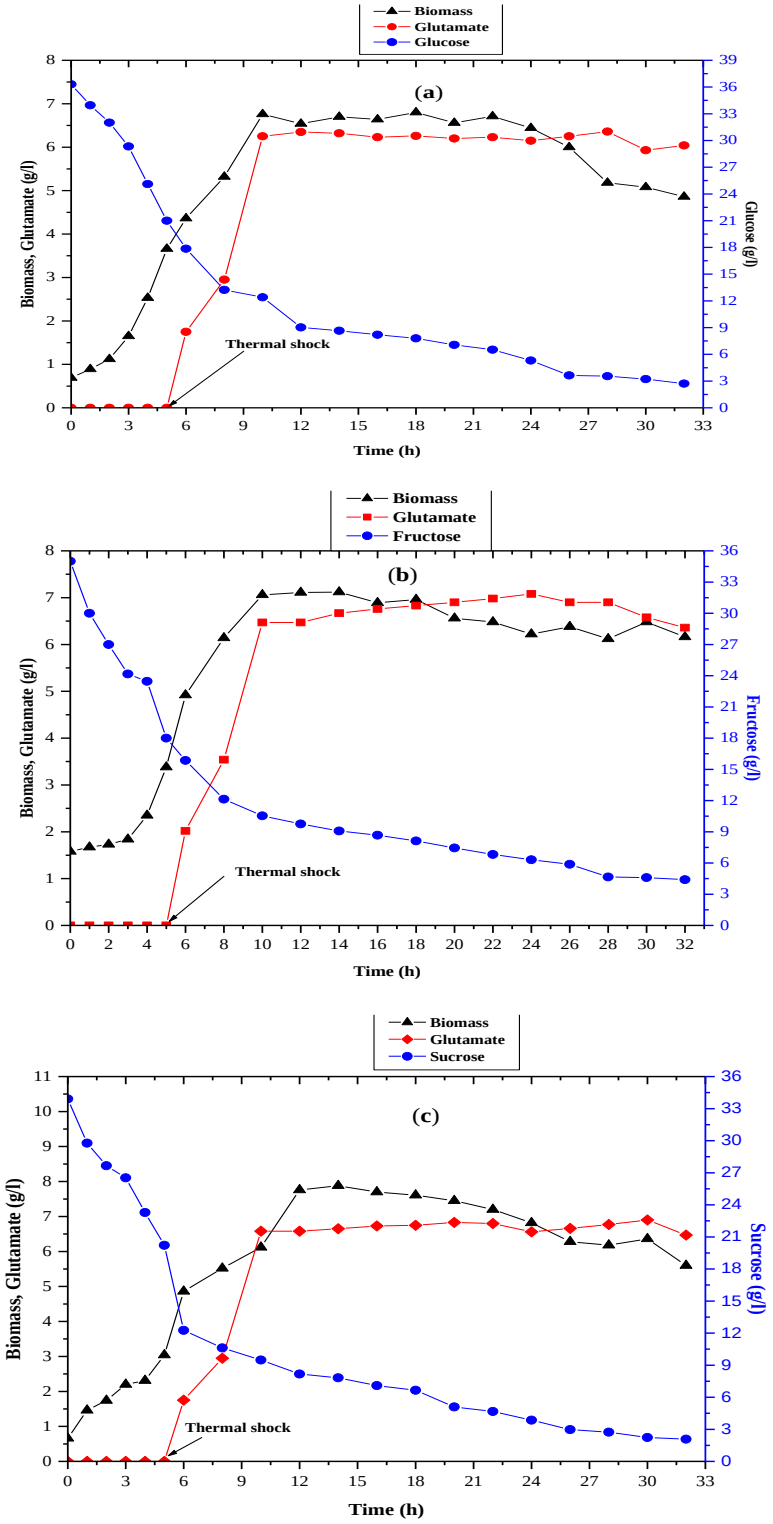

Figure 4(a, b, c). Kinetics of *C. glutamicum* 2262 in batch culture on: (a): glucose; (b): fructose; (c) sucrose

The best result of glutamic acid production is obtained on the medium containing fructose with a maximum concentration of 7.06 g/L, then sucrose and glucose with very close concentrations (6.58 and 6.36 g/L, respectively). The maximum growth rate of *Corynebacterium glutamicum* on sucrose and glucose was faster (0.29 1/h) than on fructose (0.18 1/h). The same result was obtained by Dominguez et al. (1997). Nevertheless, the sugars in each medium are consumed almost completely (Figure 4) with a low substrate consumption rate between sucrose and glucose (93.86% and 92.53%, respectively), and a lower rate for fructose (87.43%). The maximum value of biomass is reached after 10 h of culture for glucose and fructose (Figures 4a, 4b). However, for sucrose, the latter reached the maximum value after 12 h of culture (Figure 4c). As a result, the volumetric productivity of glutamic acid production (Table 3) is identical for the three media. An identical result was obtained by Georgi et al. (2005) which tested the effect of carbon sources on glutamate production on *Corynebacterium glutamicum* ATCC1032.

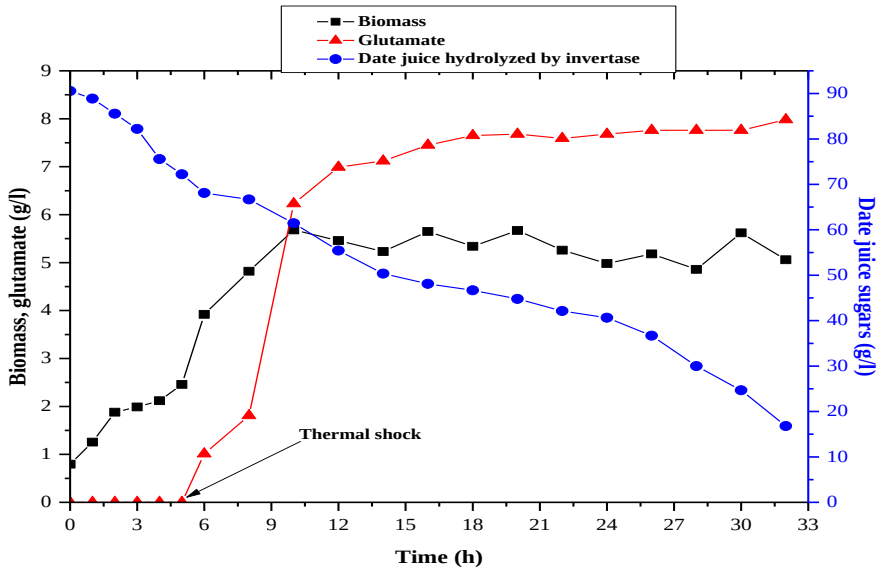

Figure 5. Kinetics of *C. glutamicum* 2262 on date juice hydrolyzed by invertase in batch culture

Table 4. Kinetic parameters of *C. glutamicum* 2262 on date juice hydrolysed by invertase in batch culture.

Carbon Source	X max (g/L)	Glutamate max (g/L)	µmax (1/h)	qs (g/g.h)	Substrate consumption rate (%)	δ (g/L.h)
Date Juice (g/L) without invertase	5.84	7.55	0.26	1.86	92.53	0.71
Date Juice (g/L) With Invertase	7.68	7.98	0.22	3.36	81.44	0.72

X: biomass; µmax: maximum specific growth rate; qs: specific rate of substrate consumption. (%); (δ): volumetric productivity of glutamic acid production.

Kinetics of Corynebacterium glutamicum 2262 on date juice hydrolysed by invertase

Fermentation in batch culture was carried out on date juice hydrolysed by invertase. The conditions for the hydrolysis of sucrose of the date juice are as follows: 8 mg of invertase dissolved in 1 mL of 0.1 M sodium acetate buffer, pH 8. The reaction is carried out at pH 4.5, at a temperature of 55°C and a stirring of 200 rpm for 40 minutes (Rebros et al., 2007). The results obtained are shown in Figure 5.

In order to interpret the results, several kinetic parameters were calculated. The main parameters are summarized in Table 4. Under these conditions, the hydrolysis of sucrose contained in the date juice seems to be beneficial for *Corynebacterium glutamicum* 2262 whether for growth whose maximum concentration reached 7.68 g/L, or for the maximum concentration of glutamate (7.98 g/L), and also the specific rate of consumption of the substrate which arrived up to 3.36 g/g.h.

Conclusion

In the present work, effect of different carbon sources on glutamic acid production *from C. glutamicum* 2262 in batch culture, were performed. It was concluded that date juice promotes the growth of bacteria and even the production of glutamic acid. Bacterial kinetics have been illustrated on media containing a single sugar: glucose, fructose, sucrose or a mixture of these three sugars. For media containing simple sugars, the best results were obtained from sucrose either for growth or for the production of glutamate (9.52 g/L and 6.58 g/L, respectively). For the medium containing mixed sugars, the concentration of glutamate produced (8.41 g/L) is higher than that of the sugars alone and of the date juice. The hydrolysis of the sucrose contained in the date juice improves the growth and the production of glutamate (7.68 g/L and 7.98 g/L, respectively) compared to that of the date juice.

References

Benyahia-Krid FA, Khemissat E, Aissaoui Zitoun O, Djafri K, Bergouia M, Meghzili B, El-Mechta L. 2021. Technological Valorization of Algerian Dates Downgraded from Deglet Nour Variety to Semi-Candied Dates in Dates Syrup. Food and Nutrition Sciences, 12, 627-642. https://doi.org/10.4236/fns.2021.126047

Delaunay S, Gourdon P, Lapujade P, Mailly E, Oriol E, Engasser JM, Lindley ND, Georgen JL. 1999. An improved temperature triggered process for glutamate production with Corynebacterium glutamicum. Enzyme and Microbial Technology. 25, 762-768. https://doi.org/10.1016/S0141-0229(99)00120-9

Dominguez H, Cocaign-Bousquet M, Lindley ND. 1997. Simultaneous consumption of glucose and fructose from sugar mixtures during batch growth of Corynebacterium glutamicum. Applied Microbiology and Biotechnology. 47, 600-603. https://doi.org/10.1007/s002530050980

Dominguez H, Lindley ND, 1996. Complete sucrose metabolism requires fructose phosphotransferase activity to ensure phosphorylation of liberated fructose. Applied and Environmental Microbiology 62, 3878-3880. https://doi.org/10.1128/AEM.00573-18

Dong Y, Xuexiu J, Mengyuan D, Feng H, Feng S, Hiroki T, Jiří JK. 2020. Life cycle assessment of cleaner production measures in monosodium glutamate production: A case study in China. Journal of Cleaner Production. 270, 122-126. https://doi.org/10.1016/j.jclepro.2020.122126

El Arem A, Flamini G, Saafi EB, Issaoui M, Zayene N, Ferchichi A, Hammami MH. 2011. Chemical and aroma volatile compositions of date palm (Phoenix dactylifera L.) fruits at three maturation stages. Food Chemistry. 1744–1754. https://doi.org/10.1016/j.foodchem.2011.02.051

Eggeling L, Bott M. 2005. Handbook of Corynebacterium glutamicum, Florida: CRC press. https://doi.org/10.1201/9781420039696

Ganguly S, Banik AK. 2012. Studies on the microbial production of L-glutamic acid. International Journal of Deccan Pharma and Life Sciences 3(2), 37–77.

Georgi T, Doris R, Wendisch V. 2005. Lysine and glutamate production by Corynebacterium glutamicum on glucose, fructose and sucrose: Roles of malic enzyme and fructose-1,6-bisphosphatase. Metabolic Engineering 7, 291-301. https://doi.org/10.1016/j.ymben.2005.05.001

Hideaki Y, Masayuki I. 2013. Corynebacterium glutamicum, biology and biotechnology. Ed. Munster: springer. https://doi.org/10.1007/978-3-642-29857-8

Liming D, Yougzhi L, Ping W, Zhihe F, Ning D. 2018. Cleaner production of monosodium glutamate in China. Journal of Cleaner Production. 190, 452-461.https://doi.org/10.1016/j.jclepro.2018.04.098

Mouffok A, Bedaida IK, Nancib A, Nancib N, Goergen JL, Boudrant J. 2012. Sequential optimization approach for enhanced production of glutamic acid from Corynebacterium glutamicum 2262 using date juice. Journal of Biotechnology Bioprocess Engineering. 17, 795-803. https://doi.org/10.1007/s12257-011-0486-8

Mouffok A, Nancib A, Boudrant J, Nancib N. 2021. High production of L-glutamic acid from date juice extracts by Corynebacterium glutamicum using fed-batch cultures: pulsed and continuous feeding modes. Acta Scientifica Naturalis 8(1), 37–50. https://doi.org/10.2478/asn-2021-0004

Nancib N, Ghoul M, Larous L, Nancib A, Adimi L, Remmal M, Boudrant J.1999. Use of date products in production of the thermophilic dairy starter strain Streptococcus thermophilus. Bioresours. Technology. 67, 291–295. https://doi.org/10.1016/S0960-8524(98)00115-1

Péquignot C, Dussap CG, Pons A, Gros JB. 1997. Intra- and extracellular concentrations of glutamate, lactate and acetate during growth of Corynebacterium glutamicum on different media. Journal of Industrial Microbiology & Biotechnology 18, 312-318.https://doi.org/10.1038/sj.jim.2900386

Rebros M, Rosenberg M, Mlichová Z, Krištofíková L. 2007. Hydrolysis of sucrose by invertase entrapped in polyvinyl alcohol hydrogel capsules. Food Chemistry, 102, 784-787. https://doi.org/10.1016/j.foodchem.2006.06.020

Shaik YP, Mir NA, Hajera T, Mazharuddin KM. 2011. Comparative studies on production of Glutamic acid using wild type mutants immobilized cells and immobilized mutants Corynebacterium glutamicum. International Journal of Engineering Science and Technology 3(5), 3941 – 3949. https://www.researchgate.net/.../267833302

Tavakkoli M, Hamidi-esfahani Z, Hossein azizi M. 2009. Optimization of Corynebacterium glutamicum glutamic acid production by response surface methodology. Food Bioprocess Technol, 10, 7-9. https://doi.org/10.1007/s11947-009-0242-7

Teramoto H, Inui M, Yukawa H. 2011. Transcriptional regulators of multiple genes involved in carbon metabolism in Corynebactreium glutamicum. J. Biotechno. 154, 114–125. https://doi.org/10.1016/j.jbiotec.2011.01.016

Von der Osten CH, Gioannetti C, Sinskey AJ. 1989. Design of a defined for growth of Corynebacterium glutamicum in which citrate facilitates iron uptake. Biotechnol. Lett. 11, 11-16. https://doi.org/10.1007/BF01026778