Cost-effective bioethanol production at low content of nitrogen source from carob syrup

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Abstract

Ethanol, as biofuel, has received great interest in the latest decades due to its potential as an alternative transport fuel. Nowadays, ethanol can be produced through fermentative processes, using sugar rich agricultural raw material and it may have a significant role in reducing environmental impact of fossil fuels. The increasing use of renewable energy sources has been the impetus to search for novel cost-effective carbon sources for biofuel production [1]. In this context, residues of carob (*Ceratonia siliqua L.*) pod appears as a cheap carbon source for biological production since there is a large content of carbohydrates, such as glucose, fructose and sucrose [2]. Carob is a perennial leguminous tree and is a substantial component of the Mediterranean vegetation and has an important role in the economy of south of Portugal. In fermentation processes for bioethanol production, *Saccharomyces cerevisiae* yeasts have been widely used due to its considerable tolerance to high concentrations of ethanol and sugar content and low pH values [3]. Ethanol is produced as a secondary metabolite and appears in stationary phase. Elements such as carbon and nitrogen are both required in yeast metabolism. Nitrogen plays an important role in protein biosynthesis and enzymatic functions and its input affects yeast metabolism. It can be supplied to *S. cerevisiae* growth medium as ammonium, nitrate or organic compounds, though it's in ammonium ion shape that yeasts assimilate nitrogen [4].
The aim of this study was to optimize nitrogen content for *S. cerevisae* fermentation medium to produce ethanol, from sugar-rich carob extract. Organic and inorganic sources of nitrogen were tested at different concentrations in order to assess cellular growth and ethanol production. Studies were carried out with a high ethanol tolerant *S. cerevisae* strain using carob syrup as carbon source at 250 g/L of total sugar concentration. At first, two concentrations of peptone, 20 g/L and 5 g/l, supplemented with yeast extract, were tested in 3L stirred tank reactor at 30 ºC, 250 rpm and low aeration rate with the same initial cell concentration

Table I – Kinetic parameters of ethanol production from carob extract (250 g/L sugars) for different concentrations of nitrogen source where µ is specific growth rate and $Y_{P/S}$ is ethanol/substrate yield.

Nitrogen source (peptone)	Yeast Extract (g/L)	µ (h^{-1})	Maximum Ethanol Concentration (g/L)	$Y_{P/S}$ (g ethanol/g subs)	Ethanol Productivity (g/L.h)
20 g/L	10	0.156	110.6	0.46	1.80
5 g/L	3	0.132	117.9	0.50	1.57

After 72 hours of fermentation, ethanol production reaches 117.9 g/L for 5 g/L of peptone, achieving a 0.50 g of ethanol per g of substrate yield, close to theoretical yield of 0.51, and for 20 g/L of peptone a

concentration of 110.6 g/L of ethanol was obtained with a yield of 0.46 g of ethanol per g of substrate (table I). The decrease of nitrogen source from 20 g/l to 5g/L and yeast extract from 10 g/l to 3 g/l not only did not affect ethanol production, but furthermore a slightly higher ethanol production and yield was observed which represents an enhancement in the cost-effectiveness of this process. A smaller specific growth rate of 0.123 h^{-1} , was found due to the less amount of nitrogen in the medium available for cellular growth.

A second study was carried out where different nitrogen sources were added to carob extract with 230 g/L of total sugar. This experiment was performed in shake flasks at 30 ºC, at 150 rpm and initial concentration of $1x10^7$ cells/mL of *S. cerevisae*.

Table II – Kinetic parameters of ethanol production and cellular growth for different nitrogen sources. YEP (3 g/L yeast extract and 5 g/L peptone), ammonia sulfate (3 g/L) or with ammonia nitrate (3 g/L) and urea (3g/L).

Nitrogen source	**µ (h^{-1})**	**Maximum Ethanol Concentration (g/L)**	**$Y_{P/S}$ (g ethanol/g subs)**	**Ethanol Productivity (g/L.h)**
YEP	0.068	104.3	0.501	1.372
Ammonia sulfate	0.066	85.5	0.444	0.910
Ammonia Nitrate	0.060	88.3	0.467	0.932
Urea	0.078	107.3	0.497	1.412

In this case, media supplemented with ammonia sulfate (3 g/L) or with ammonia nitrate (3 g/L) showed similar low concentration of produced ethanol, comparing with those supplemented with YEP (3 g/L yeast extract and 5 g/L peptone) and urea (3g/L). This last supplement is an organic compound which provided, in these conditions, a maximum ethanol production of 107.3 g/L within 76 hour of fermentation, with a $Y_{P/S}$ of 0.497 g of ethanol per g of substrate. Kinetic parameters of cellular growth showed that *S. cerevisae* better assimilates organic nitrogen than inorganic, which was already demonstrated by Yue *et al* [4]. These results offer a great advantage in means of sustainability of ethanol production from carob extracted carbohydrates, since there is a cost benefit in using urea at 3 g/L as nitrogen source.
Cost-effectiveness of this 2nd generation ethanol production process is based on using cheap carob pulp residues as carbon source and a low amount of nitrogen source (peptone, 3g/l) or urea (3g/L).

References

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