High ethanol productivity by fermentation of concentrated industrial substrates using ethanol-tolerant *Saccharomyces cerevisiae* strains

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Growing industrial development makes imperative to find environmentally sustainable energy sources. Biofuels have gained popularity in the last decades, especially due to the fossil fuels depletion and the increasing environmental concern. Of the many fossil fuels' alternatives, ethanol has been highly used and studied, since it can be added to fuels for combustion engines to levels of up to 20% without any modification to the engine (MDA, 2007). In order to make ethanol production more economical, a high productivity bioprocess is necessary. Ethanol productivity can be improved by using highly concentrated substrates. However, ethanol is well known as a toxic metabolite for yeast cells, which raises a serious problem. Thus, strains that can grow well under high ethanol stress condition are highly desirable. This work aims to select and characterize *Saccharomyces cerevisiae* strains with improved ethanol tolerance. Moreover, it aims to evaluate the feasibility of industrial residues as fermentation media and to optimize the composition of such media.

Screening for strains or mutants with high ethanol tolerance has been often performed by viability observation on ethanol-containing agar plates (Carrasco et al., 2001) and by growth rates determination on ethanol-containing medium (Hirasawa et al., 2007). Although these and other similar techniques are easy and fast, they screen for the ability to grow under high ethanol stress rather then for fermentation ability in these conditions. Thus, selection must be conducted under conditions similar to those faced by yeasts in industrial processes. In the present work, strains were selected for ethanol tolerance in batch fermentations of high-glucose media, which causes osmotic (sugar) stress in the beginning and increasingly ethanol stress towards the end of the fermentation.

Three *Saccharomyces cerevisae* strains were tested for ethanol production and tolerance, from which CA 116 and RL 11 are industrial strains isolated from Brazilian "cachaça" fermentation and BY 4741 is a laboratorial strain. The most ethanol-tolerant strain was able to ferment 300 g/L glucose, producing up to 17.4 % (v/v) of ethanol in trials carried out in anaerobic shake-flasks.

Aiming to develop an inexpensive and highly fermentable industrial based medium, corn steep liquor (CSL) has been added to high glucose solutions, as sole nutrients source in replacement of both peptone and yeast extract. CSL), a major by-product of corn starch processing, is a cheap and readily available source of proteins, amino acids, minerals, vitamins, reducing sugars (such as dextrose), organic acids (in particular, lactic acid), enzymes, and elemental nutrients (such as nitrogen) (Aktar et al., 1997; Amartley et al., 2000; Keller and Heckman, 2006). Although its primary uses are as feed supplement for ruminants and nutrient source for poultry (Akhtar et al. 1997; Keller and Heckman, 2006), CSL has been used in many works as a rich and effective nutrient supplement, including for ethanol production (Lawford, 1997; Amartley et al., 2000; Carvalheiro et al., 2006). Likewise, the results obtained have confirmed the feasibility of CSL as peptone and yeast extract substitute, since supplementation of 300 g/L glucose medium with CSL concentrations around 90 - 110

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g/L has resulted in fermentation performance similar to that observed in YP medium with the same glucose concentration.
Recent studies have reported ethanol titres as high as 19 % v/v (Alfenore et al., 2002; Cot et al., 2007). However, these values have been achieved in fed batch processes with exponential vitamins feeding, besides other controls such as pH regulation and constant glucose concentration. In the current work, fermentations were carried out in simple anaerobic shake-flasks without process control; thus, higher performance may be achieved by process improvement.
In order to design sustainable bioethanol production processes it is important to understand which factors contribute for a higher microbial ethanol tolerance, as well as to design fermentation media totally based in industrial by-products or other cheap resources.

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