**Co-Culture Fermentation Strategy for Bioethanol Production from Mixed Sugars.**

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**Highlights**

* A mixed sugar composition (C5 and C6) from sugarcane bagasse hydrolysate diluted with molasses was evaluated.
* Hexoses and xylose-fermenting yeast were cultivated together in a single batch fermentation.
* Co-culture strategy was related to upper xylose consumption and bioethanol productivity.
* Maximum productivity was achieved during co-culture with *S. cerevisiae* and *S. passalidarum*.

**1. Introduction**

Techniques for fermenting mixed sugar in a single process have practical significance since they could improve process economics by shortening fermentation times, and make it possible to improve ethanol yield through complete sugar utilization from sugarcane biomass. In this context, the co-culture strategy could be an alternative process, where *S. cerevisiae* effectively ferments glucose, and the remaining xylose can be fermented by xylose-fermenting species, such as *Scheffersomyces stipitis* and/or *Spathaspora passalidarum* [1,2]. The conceptual idea is the use of first generation (1G) ethanol plants as the host for second generation (2G) ethanol technologies, since these mills have a surplus of electricity, steam, water, bagasse and others facilities. Once proved technically and economically feasible, the proposed fermentation strategy will offer a sustainable and enhanced business opportunity for the sugarcane industry.

**2. Methods**

**2.1. Yeast strains and agro industrial raw materials**

Xyloses-fermenting yeasts: *Scheffersomyces stipitis* Y-7124 (SS) and *Spathaspora passalidarum* Y-27907 (SP) were obtained from the ARS Culture Collection. Hexoses-fermenting yeasts: *Saccharomyces cerevisiae* (SA), wild-type, provided by Santa Adélia Sugar Mill, (SP, Brazil) and *Saccharomyces cerevisiae* (acB11) (respiratory-deficient mutant) (SpC), kindly provided by Dr. Mario Barros (State University of São Paulo, São Paulo, Brazil).

Sugarcane molasses (g.L-1): 414.31 sucrose (65%), 143.41 glucose (22.5%), and 79.68 fructose (12.5%), corresponding to 637.41 of total reducing sugars (TRS). Sugarcane bagasse (hemicellulosic hydrolysate): obtained after diluted sulfuric acid pretreatment (145 °C, 12 min, 0.5% (v/v) H2SO4, 1:10 (w/v)) and evaporation step (to provide partial detoxification and sugar concentration). The final composition was (g.L-1): 94.4 xylose, 12.2 glucose, 8.0 arabinose, regarding reducing sugar; and 5.5 acetic acid, 0.3 formic acid, 0.07 HMF, 0.05 furfural, regarding inhibitors.

**2.2. Co-culture fermentation strategy**

Co-cultures experiments were performed using hexoses and xyloses-fermenting yeasts in a single process (cell ratio 1:1, 20% of inoculum size). Batch runs were carried out (150 rpm, 28°C, pH 6.0, until sugar exhaustion) using a ratio of mixed sugars of 50:50 (w:w) from each carbon source (hemicellulosic hydrolysate and sugarcane molasses), ranging from 50 to 100 g.L-1. The mixture with molasses was proposed in order to improve the medium composition and reduce inhibitors concentrations. Experiments were carried out in duplicate. Periodic monitoring was carried out to assess fermentation performances (cell density by OD; sugar, alcohols and organic acids analyses using HPLC and Dionex apparatus) [3].

**3. Results and discussion**

The results are shown on Table 1. In all experiments, hexoses content was firstly utilized. Xylose utilization was slower, probably because of O2 competition and faster ethanol accumulation by *Saccharomyces* species*.* In order to investigate the O2 supply, co-cultures with a respiratory-deficient mutant of *S. cerevisiae* (ScP) were performed. During co-culture with SS+ScP, maximum ethanol titer and yield were achieved, even with high acetic acid concentrations (major inhibitor release after bagasse pretreatment). On the other hand, sugar uptake for mutant species were lower compared to wild-types, which led to reduced ethanol productivity.

**Table 1.** Co-culture fermentation with hexoses and xylose-fermenting yeast using a mixed sugar composition.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Co-culture** | **Sinitial**  **(g.L-1)** | **Pmax**  **(g.L-1)** | **Yps**  **(g.g-1)** | **Qpmax**  **(g.L-1.h-1)** | **Acetic acid**  **(g.L-1)** | **E**  **(%)** |
| SS + SA | 50 | 24.3 | 0.486 | 1.10 | 1.26 | 100 |
| 75 | 27.0 | 0.411 | 1.21 | 1.75 | 84.7 |
| 100 | 24.3 | 0.458 | 1.37 | 2.21 | 94.4 |
| SS + ScP | 50 | 17.8 | 0.311 | 1.06 | 1.42 | 64.1 |
| 75 | 31.1 | 0.391 | 0.84 | 2.25 | 80.6 |
| 100 | 49.3 | 0.472 | 0.60 | 3.26 | 97.3 |
| SP + SA | 50 | 24.8 | 0.491 | 3.75 | 1.15 | 100.0 |
| 75 | 27.9 | 0.381 | 4.05 | 1.72 | 78.6 |
| 100 | 30.2 | 0.296 | 4.44 | 2.26 | 61.0 |
| SP + ScP | 50 | 16.7 | 0.338 | 1.01 | 1.23 | 69.7 |
| 75 | 23.1 | 0.304 | 0.94 | 1.89 | 62.7 |
| 100 | 37.2 | 0.371 | 0.79 | 2.26 | 76.5 |

**4. Conclusions**

The co-culture combinations evaluated here were able to consume all reducing sugars. Considering the co-culture strategy (SS+ScP) a significant increase in ethanol concentration (49.2 g.L-1) was achieved, obtained when a mutant strain of *S. cerevisiae* (respiratory deficient) and *S. stipitis* were cultivated together. Furthermore, co-culture fermentation using wild-types strains (*S. cerevisiae*, *S. passalidarum*) showed the highest ethanol productivity (Qp = 4.44 g.L-1.h-1), which could be a good strategy to accelerate bioethanol production from lignocellulosic biomass.

**References**

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