Simultaneous saccharification and fermentation of lignocellulosic waste material for *second generation* ethanol production

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Currently, bioethanol is produced at industrial scale from sugar and starch; however, bioethanol production systems evidence several concerns about competition with food and feed supplies.[1,2] Alternatively, lignocellulosic biomasses such as agricultural wastes, woody biomasses, and lignocellulosic energy crops, today are expected to be the new *second generation* feedstock for bioethanol production because do not compete with food sources.[3,4] Nowadays, industrial bioethanol production is mainly focused on corn, wheat and sugarcane, as well as on highly abundant agricultural wastes. Lignocellulose-containing biomass is mainly composed of hemicellulose (five carbon polymers), cellulose (six carbon polymers) and lignin (phenol polymers) and therefore has to be pre-treated prior of its use in ethanol production by yeast. The use of residual biomass for bioethanol productions has the added advantage of transforming a waste material into a high-value product.[5] The hydrolysis of celluloses and hemicelluloses to hexoses and pentoses is generally achieved by the addition of several different enzymes such as cellulases and hemicellulases.[6] In this study pineapple wastes, a material rich in sugars and lignocellulosic components, were assayed with the purpose of obtaining a valuable product from the residues of the juice and canning industries. Pineapple wastes, enclosing fruit skin and core, were homogenized in a fruit blender. The resulting homogenate, with a dry matter content of 14% (w/w), was diluted with water to a 9% dry matter in a working volume of 1.5 L, and immediately treated at 100°C for 10 min under continuous mixing to inactivate endogenous hydrolytic enzymes and reduce in the same time any microbial spoilage. No further sterilization procedure was adopted. Simultaneous saccharification and fermentation (SSF) was carried out adding together a commercially-available cocktails of cell-wall degrading enzymes and active *Saccharomyces cerevisiae* NCYC 2826 inoculum (approximately 10^7 cells per mL) to the substrate. Fermentation parameters were: 30°C, pH 4.5 and constant stirring at 200 rpm. CO_2 evolution was measured during the fermentation and representative samples of the fermenting substrate were taken at regular intervals. For each sample, ethanol, glycerol, soluble and insoluble sugars were evaluated using GC and HPLC methods. Moreover, total protein determination by Kijeldahal method and Klason lignin were carried out. Substrate initial fibers and soluble sugars were 23.9 and 42.2% respectively (Table 1); at the beginning of the fermentation Glucose and Xylose were the most abundant neutral monosaccharides followed by Galacturonic acid, Arabinose, Galactose and Mannose, with smaller proportions of Rhamnose and Fucose. The main sugars in the soluble fraction were Glucose and Mannose; only small amounts of Galacturonic acid and Galactose was detected. By 21 hours soluble sugars and fiber utilization by the yeast, as well as ethanol production, stopped (Figure 1). The highest ethanol production was 3.7%, reach-

Table 1. Dry matter (DM), fiber and soluble sugars on dry matter, pH, EtOH amount and theoretical yield (TY*), glycerol, protein lignin and ash for SSF process.

	DM%	fiber in DM, %	soluble sugars in DM,%	pH	ethanol (V/V) %		Glycerol %	Protein %	Lignin %	Ash %
					amount	TY %				
Initial	9.0	23.9±2.0	42.2±3.0	4.5	0.1± 0	0.0± 0	0.0± 0	4.1±0.2	3.6±1.0	0.5±0
final	1.7	3.4±0.5	7.5±1.1	3.3	3.7±0.1	96 ± 1	1.0±0.1	17.2±1.5	7.9±0.8	0.6±0

* TY (theoretical yield represents the max ethanol yield: 0.511 g alcohol per 1.0 g glucose.

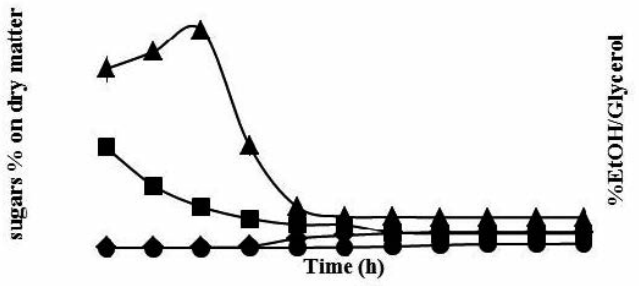

Figure 1. Fiber % (square), soluble sugar % (triangle) calculated on initial dry matter, EtOH % (diamond) and glycerol % (circle) in pineapple waste fermented by *Saccharomyces cerevisiae* NCYC 2826 during simultaneous saccharification and fermentation (SSF).

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ing a 96% of the theoretical yield (TY). Data about fiber, soluble sugars, ethanol, glycerol, protein, lignin and ash are reported in Table 1. Though the ethanol yield obtained appears rather low, due of course to the low sugar content in the starting material, SSF of pineapple wastes could be attractive since TY, calculated on dry matter loss, reached up 96%, making these wastes an excellent raw material for ethanol production by *S. cerevisiae* NCYC 2826. Moreover substrate resulting from the fermentation process is enriched in protein and lignin, suitable, after separation, for feed and further fuel production respectively.

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