Production of alcohol by simultaneous saccharification and fermentation of low-grade wheat flour using different microorganisms
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Keywords: low-grade wheat flour, alcohol, Saccharomyces spp., Zymomonas spp.

Introduction
In Brazil, the total amount of wheat flour produced in the year 2000 was about 6.8 million ton (FIBGE, 2001), from which nearly about 5% represents the amount of low-grade wheat flour produced.

Various authors have been utilizing wheat by-products, such as bran, for different purposes, e.g. as a source of dietary fiber (Miguel et al., 1999), but as for now we have found no references about low-grade flour as substrate for fermentation.

In this study two different samples of low-grade flour (namely, LG1 and LG2) and two different types of microorganisms were used to produce ethanol, by simultaneous saccharification and fermentation. The overall purpose was to produce alcohol using wheat by-products as substrate.

Material and Methods
Raw material: Two different samples, namely Low-grade flour 1 (LG1) and Low-grade flour 2 (LG2), were provided by Nisshin Flour Milling Co., Japan. In LG1 the average starch content (15.6%) was higher than in LG2 (10.4%).

Microorganisms: Two different types of microorganisms were utilized separately for fermentation: 1) Saccharomyces cerevisiae NBRC2114. 2) Zymomonas mobilis NBRC13758.

Enzymes: The hydrolysis was conducted in two steps. For the liquefaction, α-Amylase (EC 3.2.1.1. Sigma, USA) was used. The saccharification, second part of hydrolysis, occurred simultaneously with fermentation; for saccharification the enzyme amylglucosidase (EC 3.2.1.3. Sigma, Japan) was added, together with the inoculum.

Liquefaction: Initially 1L of slurry containing 10% (w/v) of low-grade wheat flour was prepared in distilled water, pH 7; α-amylase was added (400 U.g-substrate⁻¹) and hydrolyzed at 55°C for 2h, stirred at 100 rev.min⁻¹.

Starter culture: Two different starters were used in separate: 1) S. cerevisiae pre-cultured at 28°C for 24h in YM broth; and 2) Z. mobilis pre-cultured at 35°C for 24h in a medium containing (in g.1⁻¹): yeast extract, 5; glucose, 20.

Simultaneous saccharification and fermentation (SSF): after liquefaction, the pH was corrected to 4.5, amylglucosidase (200 U.g-substrate⁻¹) and starter culture (either S. cerevisiae or Z. mobilis) were added to the mixture and the SSF was conducted at 35°C for 24 h, stirred at 100 rev.min⁻¹; in case of S. cerevisiae, anaerobic environment was assured by blowing N₂ (100 ml.min⁻¹) throughout the SSF. The pH was kept constant at 4.5. The fermentation was conducted in a 2L jar fermentor, with pH, temperature and stirrer control.

Analytical methods: Glucose, maltose and ethanol were analyzed by HPLC, as described in the literature (Neves, et al., 2002). Reducing sugars were analyzed using the 3,5-Dinitrosalicylic acid (DNS) method (Bernfeld, 1955).
Results and discussion

After 2 h of liquefaction, the sample LG1 depicted a higher potential as substrate for fermentation, since the amount of glucose produced (63 g.l⁻¹) was higher than the one produced in LG2 (37 g.l⁻¹). Figure 1 shows the time-course of SSF when S. cerevisiae was used for fermentation; in this case, the ethanol production was considerably higher when LG1 was used as substrate (57 g.l⁻¹), if compared to that of LG2 (35 g.l⁻¹). Once again the higher potential of LG1 as substrate for fermentation was confirmed, which was expected considering that the initial starch content was higher in LG1.

Figure 1 – SSF of low-grade wheat flour (left: LG1; right: LG2) using S. cerevisiae
(Symbols: ▲, glucose; X, reducing sugars; ●, maltose; ■, ethanol)

Figure 2 shows the time-course of SSF when Z. mobilis was used as substrate for fermentation; as was the case in Fig. 1, the ethanol production using LG1 as substrate (33 g.l⁻¹) was higher than that of LG2 (14 g.l⁻¹). The overall ethanol production was higher when S. cerevisiae was used as starter (Fig. 1), related to that of Z. mobilis (Fig. 2).

In all cases, after 12 h of SSF the glucose content reduced to nearly zero, remaining constant. This fact has already been mentioned (Fujii L, et al. 2001), and may be explained by the difference between the optimum temperature for amyloglucosidase activity (55°C) and yeast growth (35°C).

References