Effect of agitation speeds on lipase production by *Yarrowia* yeasts

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INTRODUCTION

Lipase is a highly valuable compound and its use for industrial purposes has been expanding dynamically in recent years as a result of recognition of its favourable properties in almost all areas of production. It has a wide range of usability in the food-, pharmaceutical and beauty industries, oil industry, in the production of detergents, in the textile industry and in paper production. Moreover, it can also play an important role in the protection of environment and in health care. Of the commercially available lipase enzymes used in various industries, 88% are of microbial origin, 8% are of animal origin and only 4% are of plant origin (Martínez-Martínez *et al.*, 2017). *Yarrowia lipolytica* is one of the most extensively studied yeast species, known for its remarkably high lipolytic and proteolytic activity. In the last years some novel species belonging to the *Yarrowia* genus were described (Nagy *et al.*, 2013), and some of them also have the ability to produce lipase and other valuable compounds.

OBJECTIVES

The aim of the research program is to investigate the lipase production of *Yarrowia* yeasts. Less studied *Yarrowia* yeasts were used in the work. The focus was on increasing lipase production, and the effect of oxygen concentration was studied.

MATERIALS AND METHODS

Strains: Y. *lipolytica* 1/4, *Y. lipolytica* 854/4, *Y. divulgata* NCAIM Y.02062, *Y. divulgata* 5257

Medium: The medium for lipase fermentation contained 20 g/L glucose, 6.4 g/L peptone, 10 g/L yeast extract, 1% olive oil and 0.05% Tween 80.

Conditions : 28 °C, 7 days, 100-160 rpm. Enzyme fermentations were initiated with 5 v/v% 24-hour inoculum cultures.

Determination of extracellular lipase production: the absorbances were determined spectrophotometrically at 405 nm. One unit (U) of lipase activity was defined as the amount of enzyme that releases from p-nitrophenyl laurate 1 µM of p-nitrophenol per minute (pH 7.2, 37°C).

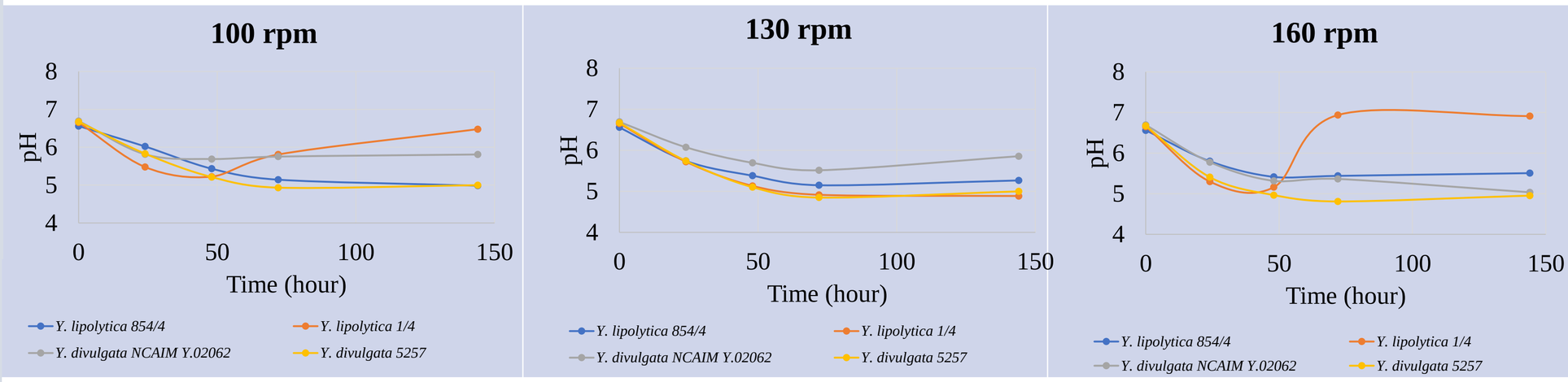
RESULTS

During the fermentation, the change in pH was monitored, as shown in Figure 1. From the values it can be concluded that for most strains pH decreased continuously over time at almost all settings. An exception is the shaking speed of 100 and 160 rpm in the case of *Y. lipolytica* 1/4, where an increase was observed after 48 h. This phenomenon is probably related to cell lysis.

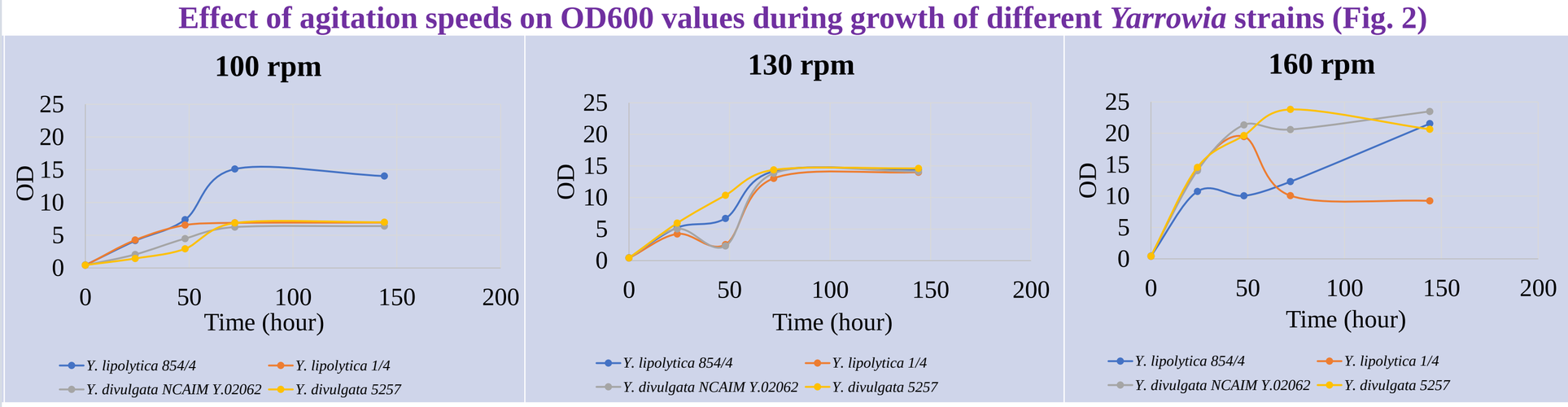
On the basis of the OD values (Fig. 2), the growth of the cells was observed all the tested agitation rates.

Based on the activity values (Fig. 3), the best producing strain at all tested shaking speeds was strain *Y. lipolytica 854/4*. The most effective speed for all strains was 130rpm, since lipase production was more efficient at this speed, with all four yeasts producing at least two to three times more enzyme than at the other two speeds. This result is also supported by data from the literature. During their research, Veerapagu *et al*. (2013) concluded that shaking is necessary for lipase biosynthesis, as the enzyme is not produced in a stationary state (without shaking), and also found that increasing the shaking speed by a reasonable amount results in an increase in lipase production. It is noticeable that there is a correlation between the enzyme activity, the pH value and the OD value. Neutral pH is most favorable for enzyme production in yeasts (Bharathi *et al*., 2018; Ramakrishnan *et al*., 2016), and neutral pH is also ideal for reproduction.

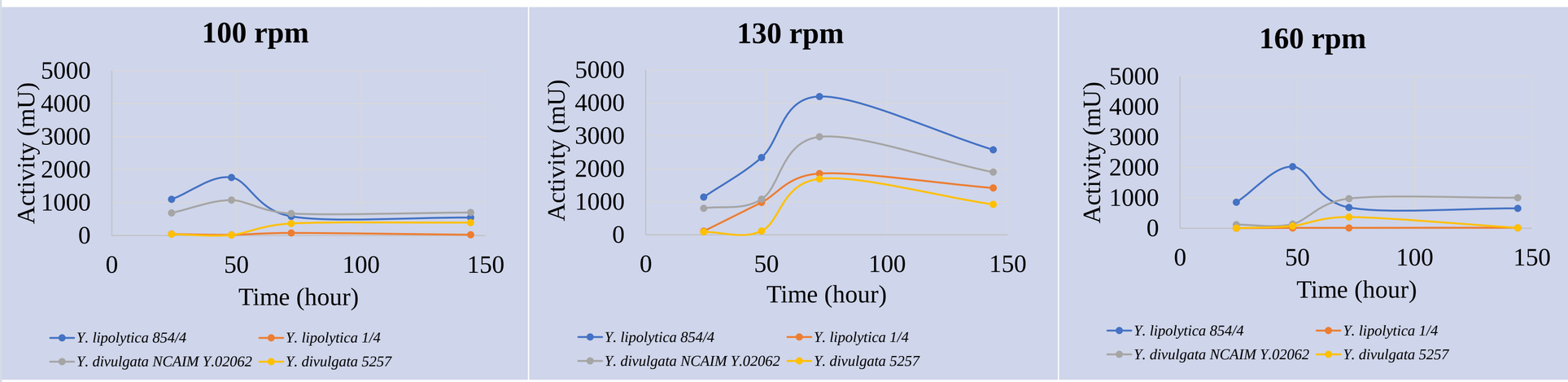
Effect of agitation speeds on pH change of different *Yarrowia* strains (Fig. 1)



Effect of agitation speeds on OD600 values during growth of different *Yarrowia* strains (Fig. 2)



Effect of agitation speeds on lipase activity of different *Yarrowia* strains (Fig. 3)



CONCLUSIONS

- It can be concluded that the pH decreased continuously over time for most strains at almost all agitation rates.
- On the basis of the OD values, the growth of the cells was observed all the tested agitation rates.
- The best lipase producing strain was *Y. lipolytica* 854/4 during all settings, but there is a significant difference between the lipase activities at different agitation rates.
- At all shaking speeds, *Y. divulgata* NCAIM Y.02062 was the better lipase producer of the two *Y. divulgata* strains.

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