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conference paper

The Use of Oxygen Uptake Rate to Optimise Air Feed Rate to a Continuous Ethanol Fermentation

Primjena brzine potrošnje kisika pri optimiranju brzine dobave zraka za kontinuiranu proizvodnju etanola

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Summary

In a continuous ethanol fermentation in a stirred tank reactor, the effect of oxygen transfer rate on the biomass concentration, specific activity and overall ethanol productivity of a thermotolerant yeast ***Kluyveromyces marxianus*** *was investigated.*

The dissolved oxygen concentration was maintained at zero. Although an increased oxygen transfer rate led to an increase in biomass concentration, the reduction in specific activity led to a reduction in overall ethanol productivity. The optimum oxygen transfer rate was zero. The maximum ethanol productivity was 1.3 mL/L h.

The ethanol productivity in the stirred tank reactor was compared with two alternative fermenter configurations:

– two fermenters in series

– poorly mixed aerated fermenter

An ethanol productivity of 3.9 mL/L h was achieved in both these systems.

Sažetak

U kontinuiranoj fermentaciji etanola u reaktoru s miješalicom ispitivan je utjecaj brzine prijenosa kisika na koncentraciju biomase, specifičnu aktivnost i ukupni kapacitet proizvodnje etanola termotolerantnog kvasca ***Kluyveromyces marxianus****.*

Koncentracija otopljenog kisika u komini održavana je na nuli. Iako je povećanje brzine prijenosa kisika dovelo do povećanja koncentracije kvaščeve biomase, smanjenje specifične aktivnosti uzrokovalo je smanjenje ukupnog kapaciteta proizvodnje etanola. Optimalna brzina prijenosa kisika bila je nula, pri čemu je postignut maksimalni kapacitet proizvodnje etanola od 1,3 mL/L h.

Kapacitet proizvodnje etanola u fermentoru s miješalicom uspoređen je s dva alternativna fermentorska uređaja:

– dva fermentora u nizu ili

– slabo miješani aerirani fermentor.

U oba sustava postignut je kapacitet proizvodnje etanola od 3,9 mL/L h.

Introduction

The production of ethanol by the thermotolerant yeast strain *Kluyveromyces marxianus* var. *marxianus* at low sugar concentration is an anaerobic process. The energy yield from anaerobic oxidation of glucose is only 3 % of that from complete aerobic oxidation to carbon dioxide and water. Reproduction will be slow under anaerobic conditions. This can lead to 'wash-out', i.e. the dilution rate is greater than the specific growth rate of the culture even at moderate dilution rates.

Alternative methods of increasing the biomass concentration in the fermenter have been investigated by the group *(1)*. These were:

(i) the use of an aerobic vessel for biomass production in series with an anaerobic ethanol production vessel;

(ii) the sedimentation of yeast biomass of the outlet stream from the fermentation vessel to produce a recycle stream with a high biomass concentration;

(iii) using a poor mixing regime with aeration which allowed sedimentation of the yeast in the fermentation vessel.

The ethanol production rate in systems (i) and (iii) above was threefold that achieved in the stirred tank reactor. This was due to a high concentration of biomass in the fermenter with a high specific activity.

The effect of the oxygen transfer rate on the ethanol productivity in the stirred tank system was investigated. It was hoped that under conditions of low aeration the ethanol productivity could be improved due to higher biomass concentrations. Maximum ethanol productivity could then be maintained through optimisation of the oxygen transfer rate.

Materials and Methods

Organism and its maintenance

The thermotolerant yeast *Kluyveromyces marxianus* var. *marxianus* was isolated from soil samples collected from Associate Distilleries, India *(2)*. It was maintained on nutrient agar slopes at 4 °C.

Shake flask and chemostat cultures

The inoculum culture was grown in 100 mL shake flasks containing 50 mL of yeast fermentation media (MYFM) which consisted of: peptone (3 g/L); yeast extract (3 g/L); KH_2PO_4 (2 g/L); $(NH_4)_2SO_4$ (2 g/L); $MgSO_4$ $7H_2O$ (1 g/L) and $MnSO_4$ (1g/L). The pH was adjusted to 5.0 using 2M KOH. A 5 mL solution of glucose (0.5 g/L) was injected into the flask prior to inoculation. The culture was incubated for 24 hours at 45 °C and 200 rpm in a New Brunswick orbital shaker. Produced culture was used to inoculate a controlled 5 L Braun Biostat-B fermenter containing 4 L of identical media. A sterile glucose solution was added to the fermenter prior to inoculation. The resulting glucose concentration was 10 g/L. The air flow rate was 4 L/min and the stirrer speed was 300 rpm. The pH was maintained at 5.0 by the addition of 2M KOH. After 24 hours the fermenter was switched to continuous operation. Two 20 L carboys containing MYFM media and glucose solution were aseptically connected to the fermenter. The solutions were pumped to the fermenter using Watson Marlow pumps giving a glucose concentration in the fermenter of 100 g/L. The dilution rate was 0.1 h^{-1}. The volume of the fermenter was maintained at 4 L by the removal of fermentation broth using a similar pump. After approximately 6 hours the agitation was reduced to 150 rpm and the air flow was switched off giving anaerobic conditions in the fermenter.

Two fermenters in series (2 STR's)

Once continuous aerobic conditions were established, a second identical fermentation vessel was connected in series (see Fig. 2). The outlet from the first vessel was fed to the second vessel via the Watson Marlow pump. The conditions in the second vessel were those described above for continuous anaerobic operation.

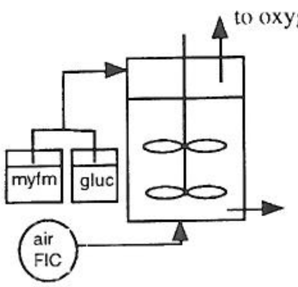

Fig. 1. Continuous stirred tank reactor (STR) with controlled aeration

Slika 1. Reaktor s miješalicom za kontinuiranu fermentaciju uz kontroliranu aeraciju

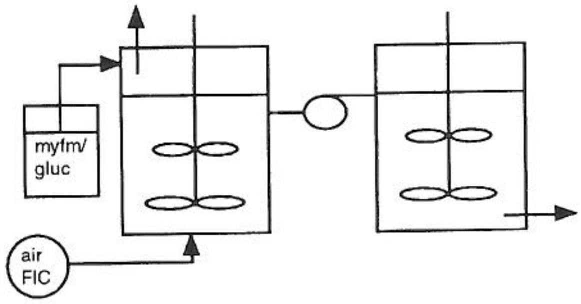

Fig. 2. Two fermenters in series

Slika 2. Dva fermentora u nizu

Continuous poorly mixed fermentation (PM STR)

For the poorly mixed fermentation a single impeller was situated at the base of the vessel. The fresh feed was pumped to the bottom and spent broth was removed from the top of the vessel. The stirred speed was reduced to 100 rpm and the air flow rate was 0.5 L/min.

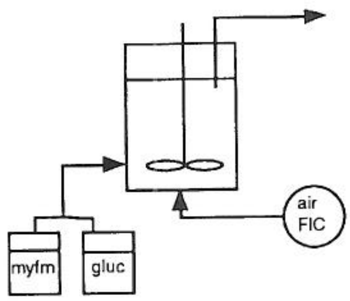

Fig. 3. Continuous poorly mixed fermentation

Slika 3. Slabo miješani aerirani fermentor

Analytical techniques

The cell dry weight was estimated from the absorbance of diluted samples of fermenter broth at 640 nm, using a standard curve. The ethanol concentration was measured using a Perkin Elmer capillary gas chromatograph.

Determination of K_La

The oxygen mass transfer coefficient, K_La for the Braun Biostat-B fermenter vessel was determined in 4 L of sterile MYFM media. The air flow rate was measured and controlled using Cole-Palmer flowmeters (0–500 mL/min). The dissolved oxygen concentration was measured using an Ingold paramagnetic oxygen electrode. The exit gas oxygen concentration was measured using a Rosemount Oxynos oxygen analyser.

Results

Increased oxygen transfer rate in an STR

– increased the biomass concentration

– decreased the specific activity.

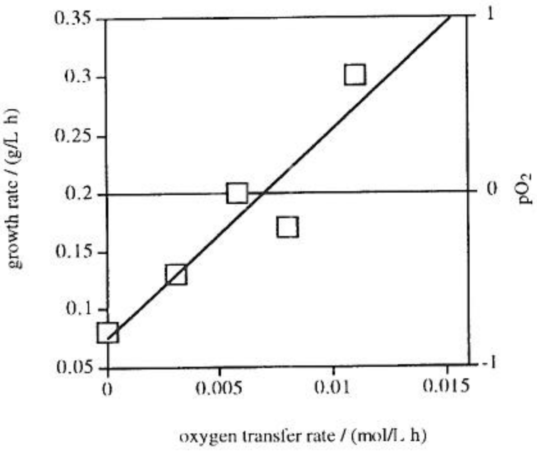

Fig. 4. Effect of oxygen transfer rate on growth rate in a stirred tank reactor

Slika 4. Utjecaj brzine prijenosa kisika na brzinu rasta u reaktoru s miješalicom

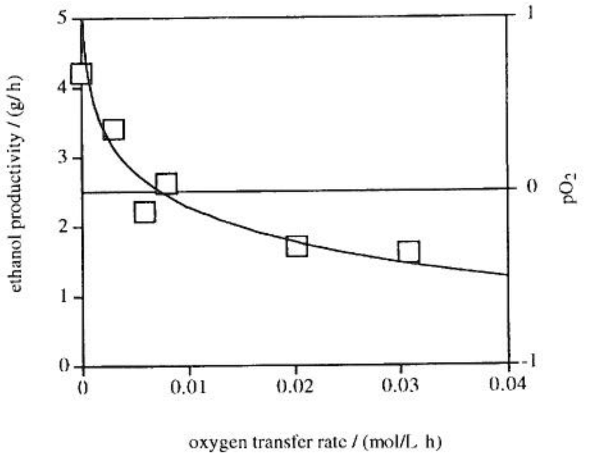

Fig. 6. Effect of oxygen transfer rate on ethanol productivity in a stirred tank reactor

Slika 6. Utjecaj brzine prijenosa kisika na proizvodnju etanola u reaktoru s miješalicom

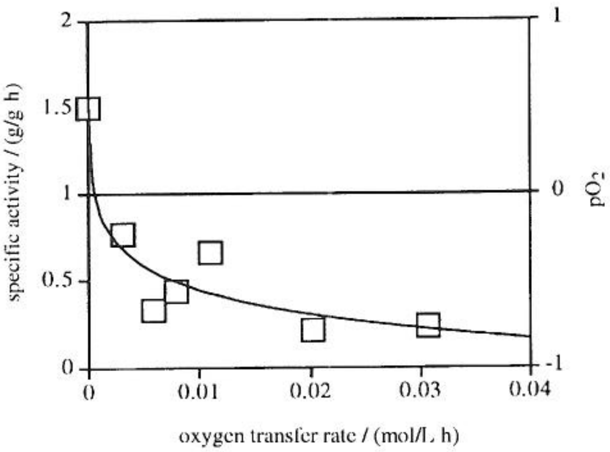

Fig. 5. Effect of oxygen transfer rate on specific activity in a stirred tank reactor

Slika 5. Utjecaj brzine prijenosa kisika na specifičnu aktivnost u reaktoru s miješalicom

The overall effect of increased oxygen transfer was to decrease the ethanol production of the system (Figs. 4–6).

Table 1 shows the ethanol productivity in the three fermenter configurations. The maximum ethanol productivity (3.9 mL/L h) was produced in the aerated poorly mixed fermenter and the two fermenters in series.

Conclusions

Increased biomass concentration due to partial aeration does not improve ethanol productivity in a continuous stirred tank reactor. This was due to the decrease in specific activity.

The use of alternative configurations to maintain a high biomass concentration during continuous ethanol production was required. The maximum ethanol production was achieved in the continuous poorly mixed reactor and by using two fermenters in series.

The use of a poorly mixed fermenter vessel would result in lower capital costs and produce higher yields of ethanol from glucose than the use of two vessels in series. This configuration was investigated further.

It was proposed that flow through the fermenter resembled plug flow behaviour. At the bottom of the fermenter oxygen was available, and growth occurred. As the yeast passed through the fermenter the oxygen was depleted and anaerobic ethanol production took place.

Further Work

The use of multiple oxygen electrodes in a larger poorly mixed vessel will be used to test the hypothesis of regions of low and high oxygen concentration.

Fig. 7 shows the proposed scheme for the control of the oxygen transfer rate during fermentation. The exit stream is monitored for biomass concentration using a 'Bugmeter' from Aber Instruments. The exit gas oxygen concentration is monitored using a Rosemount Oxynos oxygen meter. The dissolved oxygen concentration in the

Table 1. Results from fermentations in alternative fermenter configurations *(1)*

Tablica 1. Rezultati fermentacije u alternativnim fermentorskim uređajima

Fermenter	Ethanol productivity (mL/h)	Productivity/unit volume (mL/L h)	Specific activity (g/g h)	Ethanol φ/%	Yield (% theoretical maximum)
STR	5.0	1.3	1.5	1.8	70
2 STR's	32.0	3.9	0.3	4.0	75
PM STR	19.0	3.9	1.3	3.4	98

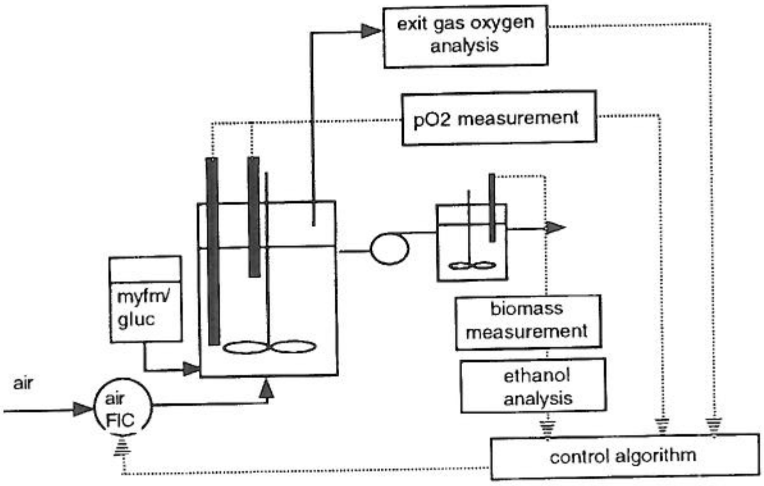

Fig. 7. Proposed mechanism for the determination of the optimum oxygen transfer rate in continuous fermentation of *K. marxianus* var. *marxianus*

Slika 7. Predloženi uređaj za određivanje optimalne brzine prijenosa kisika u kontinuiranoj fermentaciji *K. maxianus* var. *marxianus*

broth is monitored using an Ingold polarographic oxygen electrode. The broth ethanol concentration is monitored using a Perkin Elmer capillary gas chromatograph.

The oxygen transfer rate may be varied through the stirrer speed and the air flow rate. The effect on the:

- biomass concentration at different levels in the fermenter,
- biomass concentration in the waste stream, and
- ethanol concentration

would be monitored. Thus the conditions for the maximum ethanol production rate could be determined. Similar apparatus could then be employed to maintain the fermentation at optimum conditions during ethanol production.

References

1. C. Hack, I. M. Banat, Singh, R. Marchant, *Proceedings of the Conference on Fermentation Physiology*, IChemE, Brighton, July 6th 1994, pp. 7–9.
2. I. M. Banat, P. Nigam, R. Marchant, *World J. Microb. Biot* *8* (1992) 259.