



Transfer of ethanol to a solvent during fermentation in the presence of agitation

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Abstract

The aqueous phase mass transfer coefficient (K_{aq}) for ethanol transfer to the organic phase during fermentation has been successfully correlated with various system parameters in the presence of suspended yeast flocs under agitated conditions. In particular, a semi-empirical correlation of K_{aq} with the agitation speed of the stirrer, the void space in the broth, and the surface area of dead cells has been developed. In general, it has been found that K_{aq} increases with the agitation speed of the stirrer and the ratio of the void volume to the dead cell surface area. K_{aq} increases marginally though with higher incubation time at a given agitation speed of stirrer. The correlation is valid within a period of 12–30 h of incubation time. The agitation speed range is 200–300 rpm, and the ratio of void volume to dead cell surface area ratio ranged from 3.86×10^{-4} to 1.69×10^{-3} m. The fermentation time required was only 25–30 h compared to conventional fermentation systems, where it takes a minimum of 40–48 h. The organic phase mass transfer coefficient (K_{org}) is 300–500 times higher than K_{aq} . This is due to the drag of the yeast floc surfaces to the ethanol transferring eddies from the bulk to the interface.

Keywords Fermentation · Solvent · Glucose · Ethanol · Stirrer · Cell

List of symbols

A_i	Cross-sectional area of fermentor cum extractor (m^2)	D_{org}^{al}	Diffusivity of ethanol in solvent (m^2/s)
c_{aq}^{al}	Average concentration of ethanol in broth (Kg·moles/ m^3)	$\%EtOH_{aq}, \%EtOH_{org},$ $nd \%EtOH_{Total}$	Percentage of ethanol (% v/v) in aqueous, organic, and total combining both phases
c_{org}^{al}	Average concentration of ethanol in a solvent (Kg moles/ m^3)	K_{aq}	Aqueous phase mass transfer coefficient (m/h)
D_{aq}^{al}	Diffusivity of ethanol in water (m^2/s)	K_{org}	Organic phase mass transfer coefficient (m/h)
D_{IM}	Impeller diameter (mm)	K_{ov}	Overall mass transfer coefficient based on inter-phase mass transfer (m/h)
		K_I	Interfacial mass transfer coefficient (m/h)
		K_D	Distribution coefficient of ethanol between solvent and water
		N	Rotational speed of stirrer (rpm)
		Re_{org}	Organic phase Reynolds no.
		S	Surface area of the yeast cell (m^2)
		Sc_{org}	Organic phase Schmidt no.

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Sh_{org}	Sherwood no. of the organic phase
t	Incubation time in hour
V_{org}	Organic phase volume, ml
V_{aqs}	Aqueous phase volume, ml
Greek letters	
ϵ	Void volume in broth (m^3)
$\left(\frac{\epsilon}{S}\right)_m$	The ratio of void volume to the surface area of dead cells (m)
σ_d	Interfacial tension (dyn/cm)
ρ_{org}	Density of solvent (kg/m^3)
μ_{org}	Viscosity of the solvent (kg/ms)
γ	Surface tension (dyne/cm)

Introduction

The simultaneous fermentation and extraction process of ethanol production is not a new process (Sirkar 2011; Santos et al. 2020). The process of bubbling the organic solvent through the broth has been practiced so far (Ghatak 2011, Lovisa and Tran 2024 and Khoo et al. 2020). Ethanol is distilled from the organic solvent (Huang et al. 2008, 2021). However, the disadvantage of this process is the chance of emulsification caused by bubbling (Beigbeder et al. 2021; Roy et al. 2013; Terán Hilaes et al. 2017, Tian et al. 2022). This leads to the accumulation of solvent in the aqueous phase over time, causing extra toxicity (other than ethanol accumulation) in yeast cells (Roy et al. 2012, Sharma et al. 2018), thus reducing the fermentation rate.

An attempt was made (Roy et al. 2012; Munson et al. 1984; Karimi et al. 2021) to perform simultaneous fermentation and extraction in a stirred fermenter with a stirrer in both phases. Some researchers have observed that there is a great advantage of agitation in the broth to improve ethanol production (Rodmui et al. 2008; Shah et al. 2011; Kent. 2013; Palniandy et al. 2022). This is due to proper growth and microbial performance, due to improved broth rheology, uniform heat distribution, and improved mass transfer of nutrients and products/by-products through the cell wall (Pelezar et al. 1993). Transfer of glucose through the cell wall is also enhanced due to agitation according to (Sirkar 2011), caused by the generation of velocity gradients in the broth by the stirrer impellers. However, cell growth is hampered beyond some minimum agitation speed (Roy et al. 2012) due to the impellers hitting cells directly in contact with them, causing damage to cells. Also, because we have used a solvent to extract ethanol from the broth, agitation in the solvent phase also enhances overall mass transfer. The other advantage is that by distilling the solvent phase, we can

produce anhydrous ethanol. Here, we have limited emulsification due to less contact area between the phases, unlike the previous researchers (Huang et al. 2008, 2021) where they bubbled the solvent through the broth, resulting in a large surface area of bubbles. No previous researcher has studied similar fermentation with a stirrer in both phases using a solvent.

In this paper, we have presented an attempt to evaluate and correlate K_{aq} (mass transfer coefficient in aqueous phase) to system parameters that are different from the evaluation of K_{org} according to the standard correlation of Sherwood no. to Reynolds no. and Schmidt no. for a stirred system (Apreotesei et al. 2003; Karimi et al. 2021). The reason is that, in the broth, because of the high concentration of yeast flocs, the passage available for ethanol transfer to the interface from the bulk is restricted. The eddies generated by the stirrer have to pass through the void space (to reach the interface), whereby the flocs cause drag to the eddies leading to loss of momentum of the eddies, thus reducing the rate of transfer of ethanol to the interface and hence to the organic phase. The system parameters identified mainly in the present technique are the agitation speed of the stirrer and the ratio of void volume to maximum dead cell surface area. The dead cell surface area being the maximum near the end of fermentation offering the maximum drag, the correlation has been developed against this parameter to be conservative in the K_{aq} prediction.

Materials and methods

Materials

1. Distilled water: Reverse osmosis treated demineralized water was used in the experiment.
2. Ethanol: Absolute GR for analysis, Merck KGaA, specific gravity: 0.79 purity: 99.8% K35664083 604 Code: 1.00983.0511/R: 11 S: 7–16
3. Dextrose GR: E-Mark – 500G CAS No.: 50-99-7 Batch No.: MD7M570964
4. All other chemicals used as nutrients for fermentation were pure and E-Mark grade.
5. Microorganisms: *Saccharomyces cerevisiae* yeast strains (NCIM 3186) were purchased from NCIM, Pune, India.

Methods of analysis

Estimation of alcohol and glucose and cell count: The determination of ethanol was achieved by redox titration with potassium dichromate (Dubey et al. 2002), carbohydrate (glucose) by the anthrone method (Dubois et al. 1951; Dubey et al. 2002), and estimation of ethanol by GCMS (Stackler et al. 1974).

Optical density, cell count, and dry cell weight

Cell growth was measured in terms of optical density at 660 nm using a spectrophotometer (Smart Spec 3000-Biorad Laboratories) and cell count in a hemocytometer using an ETAMCAP Digi Eye 2000 photo microscope with the Diwinter software Biowizard. The measurement of dry cell weight was done using the procedure given in the literature (Shular et al. 2002). The dry cell mass concentration was determined by pipetting a definite volume of culture broth and its subsequent centrifugation. The separated wet cell mass was then dried at 80 °C for 24 h. The weight of the dry cell mass was then measured. The dry cell mass, divided by the sample volume, gave the required cell concentration.

Specification of equipment and instrument

Glass reactor

It is a batch reactor with a total volume of 300 ml and with working volume of 250 ml, fitted with an overhead REMI stirrer, Model No. RQG-121/D, VOLTS D.C-25, C. EX. no. BEU-3675 with two sets of stainless steel 316 l turbine-type impeller of four blades, evenly placed one in solvent phase and the another in aqueous phase along with digital display for stirrer speed in rpm. The reactor has a flat bottom with a drainage system at the bottom.

Reactor size: 50-mm inside diameter and 150-mm long with 20-mm overhead space.

Glass reactor specifications

- Total volume–300 ml.
- Working volume–250 ml.
- Inside diameter–50 mm.
- Outside diameter–56 mm.
- Height–150 mm.
- Overhead space–20 mm.
- Impeller diameter (D_I)–18 mm.
- Impeller blade height–17 mm.
- Impeller blade width–15 mm.

Etamcap Digi Eye 2000 photo microscope with Diwinter software Biowizard

- Hemacytometer, Neubauer improved, Superior Marienfeld, Germany
Size: Depth–0.100 mm, Area: 0.0025 mm.²
- Spectrophotometer (Smart Spec 3000-Biorad Laboratories).
- pH meter: Systronics digital pH meter 335 with PT100 temperature sensor, 4 Digit LED display range 0–14.00 pH, repeatability of $\pm 0.01\text{pH} \pm 1$ digit.

3.4 A calibrated Krüss, Germany, tensiometer based on the Du Noüy ring detachment method was used to measure the surface tension (γ) at 25 °C in mN/m.

3.5 GCMS-QP5050A Shimadzu with quadruple mass detector. Helium as carrier gas at a flow rate of 0.6 ml/min was passed through a Zebron capillary G.C. Column (Zebron 5), 30-m long, ID=0.25 mm, film thickness=0.25 μm .

Selection of solvents

Ethyl benzene was selected as a solvent to extract ethanol for the following reasons:

- Moderate toxicity (tested)
- High separation factor (97)
- Cheap and easily available

However, the distribution coefficient for this solvent was not high; rather, it was a bit low.

Sampling procedure

- The sampling was carried out aseptically using a sterile pipette at regular intervals (8 h, 12 h, 16 h, 20 h, 24 h, and 30 h).
- Separate sterile pipettes were used for separation of log hrs sampling.
- Two separate sterile pipettes were used: one set to draw samples from the aqueous phase along the side of the reactor without disturbing the interface and the second set to draw samples from the solvent phase similarly.
- Aqueous phase samples (8 h, 12 h, 16 h, 20 h, 24 h, and 30 h) were tested to estimate the glucose level, cell count, and ethanol produced.
- Organic phase samples (12 h, 16 h, 20 h, 24 h, and 30 h) were tested only for transmission of ethanol from the aqueous phase to the organic phase.

Evaluation of overall and individual phase mass transfer coefficients

$$V_{\text{org}} \frac{dc_{\text{org}}^{\text{al}}}{dt} = K_{\text{ov}} \times A_i \times \left(c_{\text{aq}}^{\text{al}} - \frac{c_{\text{org}}^{\text{al}}}{K_D} \right) \quad (1)$$

K_{ov} s have been evaluated from data of $c_{\text{aq}}^{\text{al}}$, $c_{\text{org}}^{\text{al}}$, and calculation of $\frac{dc_{\text{org}}^{\text{al}}}{dt}$ from the correlations of $c_{\text{org}}^{\text{al}}$ versus 't' at different agitation speeds where 't' is the incubation time.

And

$$\frac{1}{K_{ov}} = \frac{1}{K_I} + \frac{1}{K_{aq}} + \frac{1}{K_{org}K_D} \quad (2)$$

$$A_i = \frac{\pi(0.05)^2}{4} m^2$$

K_{org} has been evaluated from the following correlation (Apreotesei et al. 2003 and Karimi et al. 2021).

$$Sh_{org} = 54.15 Re_{org}^{1.19} Sc_{org}^{0.33} \left(\frac{D_{aq}^{al}}{D_{org}^{al}} \right)^{0.5} \times (0.341K_D^2 - 1.249K_D + 1.474) \quad (3)$$

where $Sh_{org} = \frac{K_{org}D_{im}}{D_{org}^{al}}$, $Re_{org} = \frac{ND_1^2\rho_{org}}{\mu_{org}}$, $Sc_{org} = \frac{\mu_{org}}{\rho_{org}D_{org}^{al}}$.

For the determination of the K_D , the distribution coefficient of ethanol between organic solvent and water at different incubation times corresponding to different agitation speeds, the following procedure was adopted on the basis of the experimental data as given in Table 1.

At each incubation time for a given agitation speed using the data of % EtOH in aqueous and organic phase, % EtOH_{Total} was calculated using the following equation:

$$\%EtOH_{Total} = \frac{\%EtOH_{Water} \times V_{Water} + \%EtOH_{org} \times V_{org}}{V_{Water}} \quad (4)$$

Next, from the plot of Fig. 1, K_D versus % EtOH_{Total} to start with K_D was read of the plot K_D versus % EtOH in water: (Fig. 1).

To evaluate K_{aq} at each agitation speed (rpm); as an example, K_{aq} at each incubation time was calculated using Eqs. (1) and (2) for different agitation speeds, as shown in Table 2, assuming that K_I , the interfacial mass transfer coefficient is higher than that of K_{aq} or K_{org} . This has been concluded based on a paper by England & Berg 1971. In this paper, it has been proved that in systems for transfer of surface-active solutes (here, it is ethanol), the effect of interfacial accumulation, in terms of mass transfer resistance, is negligible for contact times greater than a millisecond.

K_I could not be evaluated because we did not have any data on the interfacial concentration of ethanol in both phases. Experimental data for the calculation of K_{ov} , K_{aq} , and K_{org} are given below assuming $1/K_I \cong 0$.

Table 1 Ethanol concentration versus K_D

Ethanol in water (%v/v)	1	3	5	7	10
K_D	0.082	0.0427	0.040	0.0353	0.0294

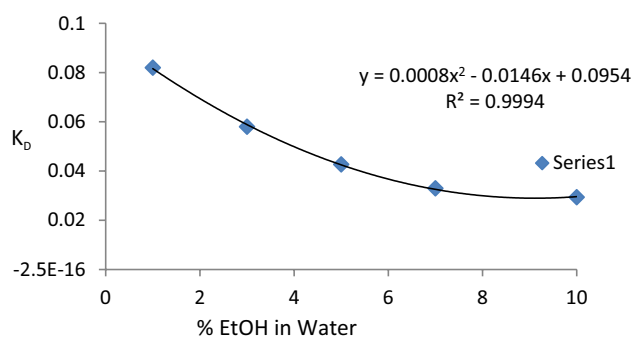


Fig. 1 K_D versus % EtOH_{Total}

Results and discussion

It is evident from Table 3 that K_{org} increases with both incubation time and the speed of stirrer. This is as per Eq. (3). However, K_{org} increases only marginally though as incubation time increases at a given speed of the stirrer. This is due to the decrease in the magnitude of K_D as the incubation time increases. This is so because with time ethanol concentration increases and as per Fig. 1, the magnitude of K_D decreases with increase in ethanol concentration. At 250 rpm, the contribution of the expression in Eq. (3) which is a function of K_D is higher at 30 h than that at 12 h. At 30 h, it is 1.39 against 1.378 at 12 h. Thus, K_{org} increases marginally from 0.1577 to 0.159 due to the change in the magnitude of K_D (Karimi et al. 2021).

K_{aq} and K_{ov} also increase with increase in the speed of the stirrer. At a higher speed of the stirrer, K_{aq} increases

Table 2 Calculated K_D at different rpm and incubation time

Agitation speed (rpm)	Incubation time (h)	c_{aq}^{al}	c_{org}^{al}	$K_D = \text{from graph}$
200	12	0.223	0.0091	0.0779024
	16	0.27615	0.01115	0.07407
	20	0.3242	0.01544	0.0706992
	24	0.3619	0.01715	0.0681733
	30	0.3619	0.01888	0.0681451
250	12	0.2213	0.0103	0.0780045
	16	0.271	0.013	0.0743985
	20	0.3225	0.017	0.0707874
	24	0.3568	0.0175	0.0685009
	30	0.3584	0.0188	0.0683749
300	12	0.20411	0.0103	0.0792666
	16	0.25214	0.013	0.0757324
	20	0.307	0.01715	0.0718354
	24	0.34133	0.01887	0.0694977
	30	0.3447	0.02058	0.0692463

Table 3 K_{ov}^{av} and K_{aq}^{av} values at different rpm

Agitation speed (rpm)	Incubation time (h)	K_D	K_{ov}	K_{org}	K_{aq}	K_{ov}^{av}	K_{aq}^{av}
200	12	0.0779024	0.000278	0.120943779	0.000244	0.000238	0.000245
	16	0.07407	0.000219	0.121346238	0.000244		
	20	0.0706992	0.000241	0.121700953	0.000245		
	24	0.0681733	0.000212	0.121967196	0.000245		
	30	0.0681451	0.00024	0.121970171	0.000245		
250	12	0.0780045	0.000382	0.157713183	0.000315	0.000307	0.000316
	16	0.0743985	0.000323	0.158206972	0.000315		
	20	0.0707874	0.00034	0.158702491	0.000316		
	24	0.0685009	0.000246	0.159016768	0.000316		
	30	0.0683749	0.000244	0.159034092	0.000316		
300	12	0.0792666	0.000467	0.195712321	0.000467	0.000453	0.000468
	16	0.0757324	0.000405	0.196313097	0.000467		
	20	0.0718354	0.000448	0.196976962	0.000468		
	24	0.0694977	0.000409	0.197375894	0.000468		
	30	0.0692463	0.000536	0.197418828	0.000469		

because eddies are thrown at higher velocity by the impellers of the stirrer such that more ethanol gets transferred from bulk to the interface through the void space of the suspension of yeast cells. This is more so due to increase in the generation of more ethanol due to higher velocity of the eddies past the yeast cells, i.e., higher fermentation rates (Palniandy et al. 2022; Rodmui et al. 2008; Shah et al. 2011); which is eventually carried by the eddies to the interface. That K_{ov} increases with the speed of stirrer is because of the increase in both K_{org} and K_{aq} . However, it varies with K_D at a given speed of the stirrer differently; sometimes increasing and sometimes decreasing. This is probably because of two factors, namely, one is the change in the magnitude of K_D , and the other is the bulk concentrations of ethanol in both the phases that are evident from Eqs. (1) and (2).

Development of the correlation of K_{aq} regarding different system parameters

From Table 4 given here, note that with an increase in agitation speed of the stirrer the number of cells per ml of aqueous phase; the broth to begin with decreases. This is because in the growth period with higher agitation speed, there is a net death of live cells in the zone within the impeller domain

caused by the impeller blades in contact with the cell wall (Sirkar 2011). The impeller blades directly hit the cell wall, causing damage to the cell wall, hence, a net loss of live cells to begin with at higher agitation speed. Because of this at higher agitation speed, near the end of fermentation when the live cells have died, the dead cell surface area is smaller. However, note that the volume of passage through which bulk of liquid containing ethanol moves toward the interface increases with higher agitation speed because the volume occupied by cells decreases with higher agitation speed. Agitation speed did not increase beyond 300 rpm for two reasons. Ethanol in general has more affinity to stay in the aqueous phase at higher concentrations because both are polar compounds. Again, this is more so for ethanol not going to the organic phase because of the aliphatic side chain of ethyl benzene which causes less affinity of ethanol. Note that although with higher concentration of ethanol more of it would be adsorbed at the interface, it would preferably stay adsorbed at the interface due to large desorption barrier (Berg 1971). Hence, the distribution coefficient decreases at higher ethanol concentration as is evident in Fig. 1. At higher agitation speed with less volume of organic solvent than that of the broth, it was observed that there was a tendency to emulsify. This is because the interfacial tension may be low

Table 4 Aqueous phase mass transfer coefficient at rpm and volume-to-surface area ratio of cells

Sl. no.	K_{aq}	Agitation speed (rpm)	Void volume (m^3)	S Surface area (m^2)	$\frac{\epsilon}{S}(m)$	Cell count (no/cc)	$f \frac{\epsilon}{S_m} (m)$
1	0.000245	200	148	3.833×10^{-1}	3.86×10^{-4}	7.73×10^9	1.48×10^{-4}
2	0.000316	250	174	1.910×10^{-1}	9.10×10^{-4}	3.81×10^9	1.74×10^{-4}
3	0.000469	300	184	1.09×10^{-1}	1.69×10^{-3}	2.17×10^9	1.84×10^{-3}

due to the adsorptive accumulation of EtOH such that the momentum of the eddies thrown by the stirrer could easily penetrate from the organic side of the interface due to low σ_d . The chance of penetration of the interface by the eddies from the broth side is less because K_{aq} is much lower than K_{org} such that the momentum of eddies gets reduced when reaching the interface due to drag by the cells. In addition, at higher agitation speed due to fewer live cells to begin with, the conversion of glucose to ethanol was less.

The mass transfer coefficient K_{aq} is dependent on the agitation speed of the stirrer, ϵ and S . This is because at higher agitation speed, the stirrer pushes liquid with higher momentum from bulk to interface. Then again, if the void volume, i.e., the passage volume increases, K_{aq} would increase because there is less volume of broth occupied by the cells. Also, as ‘ S ’ decreases at higher agitation speed, K_{aq} increases due to less drags on the surface of the total cells as the liquid flows through the volume of the void. Therefore, K_{aq} would increase with the increase in the ratio $\left(\frac{\epsilon}{S}\right)_m$.

$$\left(\frac{\epsilon}{S}\right)_m = \frac{(\epsilon/S)}{\frac{\text{No of Cells}}{2.17 \times 10^9} \times f} \quad (5)$$

In Eq. (5) ‘ f ’ is the factor by which the surface of the dead cell increases more than that of live cells due to a decrease in the size of dead cells. However, since we do not know the particle size distribution of dead cells, it is assumed that it is a multiplying factor ‘ f ’ (> 1) for the 300-rpm case. For lower agitation speed, it becomes proportionately higher due to an increase in the number of live cells that eventually die at the end of fermentation. It may be noted that although the calculation of the surface area of cells is based on individual yeast cell size, in reality, yeast cells remain as flocs of large diameter, and this diameter varies with the agitation speed of the stirrer (Brohan et al. 1984; Hu et al. 2018). Then with a higher agitation speed, we would have more surface area of the yeast flocs for a given number of yeast cells. But, since our calculation of ‘ S ’ is based on dead cells that are generally within the size range of 0.01 micron, which is much smaller than live cells, it makes no difference due to variation in yeast flocs size caused by variation in agitation speed of the stirrer.

Thus, K_{aq} has been successfully correlated to agitation speed and $\left(\frac{\epsilon}{S}\right)_m$ as given below where ‘ f ’ is built into the coefficient of $(\epsilon/S)_m$ in the correlation.

$$\ln K_{aq} = 3.62 \times 10^{-3} N + \left(\frac{\epsilon}{S}\right)_m \times 181.5 - 9.05 \quad (6)$$

In Eq. (6), K_{aq} could have been correlated using a dimensionless group, e.g., Sherwood no., Reynolds no. provided that we had viscosity data of the broth. Because of such a correlation, we also had to have void fraction and surface

area ratio of live cells and dead cells as two additional dimensionless groups as per the correlation developed. The fact that K_{aq} increased slightly with an increase in incubation time at a given agitation speed (Table 3) is because the surface area of dead cells is higher than that of live cells, with time giving more drag, but then due to the production of more ethanol and less glucose with time, μ_{broth} decreased which reduced the overall drag. This is because drag by the cells’ surface is not that high because of the soft surface of the flocs, causing a slippage of eddies that flow through the void space while being in contact with the cells’ surface to transfer ethanol from the bulk to the interface. In fact, in the correlation for K_{aq} developed, the contribution of agitation speed is significant compared to that of $\left(\frac{\epsilon}{S}\right)_m$ because higher agitation speed increases the momentum of the eddies (which are transferred from the bulk of the broth to the interface) more than the corresponding reduced drag due to the reduced surface area of dead cells at higher voidage, which is not that high due to slippage at higher agitation speed.

Conclusions

On the basis of the correlation of K_{aq} against agitation speed and void volume to surface area of dead cells ratio, it is concluded that a similar correlation may apply to simultaneous fermentation and extraction cases where the solvent may be different from the one experimented. A higher agitation speed of stirrer in the organic phase and a lower agitation speed in the aqueous phase may be desirable for higher conversion of glucose to ethanol, although this goes against the value of K_{aq} , which would decrease. However, the removal of ethanol from the aqueous phase eventually helps increase conversion because there is less toxicity due to ethanol build-up. Therefore, an optimal agitation speed of the stirrer in the aqueous phase is desirable. It is also concluded that in a stirred system with solvent, the fermentation time required is only about 25–30 h. Whereas the previous researchers reported 40–48 h in the absence of solvent, although some of them had used a stirrer in the broth. This is because fermentation occurs at a higher rate due to reduced toxicity of ethanol, which is not allowed to build up in the broth due to extraction. A better single organic solvent or a mixture of two may be desirable having a higher distribution coefficient of ethanol and a higher binary interfacial tension between the solvent and water. These are apparent from the study carried out. Furthermore, improved concepts of fermenter design are necessary to cut down fermentation time more than that achieved by us in the present study. In this respect, it may be noted that reduction of the fermentation

time achieved in the stirred fermenter used by us is due to the creation of a velocity gradient in the broth by the impellers of the rotating stirrer. Thus, the rates of diffusion of glucose and ethanol from bulk to cell wall and cell wall to bulk were faster due to the reduction of the film thickness of liquid next to the cell wall.

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Data availability Availability of all data and materials generated or analyzed during this study is included in this article. The authors want to state that there is no conflict of interest in the current work.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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