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Cooling System Economy in Ethanol Production Using Thermotolerant Yeast Kluyveromyces Sp. IIPE453

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Abstract The growth of thermotolerant/ thermophilic ethanol producing yeast and the fermentation processes using sugary substrates are exothermic processes. If the fermenters are heat insulated, the requirement of heat for maintaining the fermentation broth for ethanol production may be reduced considerably. The heat generated due to growth of thermotolerant yeast *Kluyveromyces* sp. IIPE453 was found to be 652kJ mol^{-1} at 50 ℃ using glucose as a substrate. The heat generated due to ethanol formation by *Kluyveromyces* sp. IIPE453 was found to be 132.54kJ mol^{-1} of sugar consumed or 67.84kJ mol^{-1} of ethanol produced at 50 ℃ using sugarcane molasses as substrate. This heat would be sufficient for maintaining the desired temperature, if insulated fermentation systems are used. Therefore, no additional heat would be required to maintain the temperature in fermentation process by thermotolerant yeast at 50 ℃.

Keywords: *metabolic heat, bioreactor, ethanol fermentation, thermotolerant yeast, cooling water*

1. Introduction

Most of the potential ethanologens that are in industrial use belong to mesophilic group (28-35 ℃) [1,2,3,4,5]. However, bioethanol production by thermophiles has certain advantages over mesophiles and it needs to be investigated for ethanol production. Solvent tolerance, energy savings through reduced cooling costs, higher saccharification and fermentation rates, continuous ethanol removal and the reduced risk of contamination have stimulated a search for thermophilic or thermotolerant yeasts [6,7,8,9,10]. Less energy would be required for mixing of fermentation broth containing sugar, product and cell mass and product recovery in thermophilic fermentations because of lower viscosity, surface tension, higher vapor pressure and increased solubility of organic compounds [11]. Ethanol production at high temperature is effective in tropical countries where average day-time temperatures (30-40 ℃) are usually high throughout the year [9,12].

The heat generation and its regulation accompanied by metabolic turnover is an inherent property of living systems [13,14].

$$C_6H_{12}O_6 + Microorganisms \rightarrow 2C_2H_5OH + 2CO_2 + \Delta H_r \quad (1)$$

Where, ΔH_r is heat generated due to biological reactions (kJ). The bacterium *Zymomonas mobilis* and yeast *Saccharomyces cerevisiae* are known to gain the energy required for growth and maintenance under anaerobic conditions by substrate phosphorylation processes through the Entner-Doudoroff pathway and glycolysis, respectively [15]. Theerarattananoon et al. [16] reported overall metabolic heat generation 43kJ mol^{-1} glucose consumed or 32kJ mol^{-1} ethanol produced during fermentation using *S. cerevisiae* at 25 ℃, when initial glucose concentration was kept at 300g l^{-1} glucose.

The heat loss to environment is negligible in the industrial scale bioreactors because these are operated nearly adiabatically due to much lower surface to volume ratio compared to laboratory-scale bioreactors [17], requiring huge quantity of cooling water to maintain optimum temperature. Approximately 60-70% of total chilling water load is used in bioreactors for ethanol production by using mesophiles [18]. In tropical countries, the cooling water at 28 ℃ is used to maintain the fermentation temperature at 32 ℃ in bioreactor. Heat generation by exothermic sugar fermentation (Eq.(1)) by thermophilic/thermotolerant microorganisms has an impact on the net heat balance vis-à-vis cooling water requirement in such fermentation process.

In the present paper, the metabolic heat generation on sugar consumption or ethanol production by the thermotolerant yeast *Kluyveromyces* sp. IIPE453 is reported and the advantage on cooling water requirement over *S. cerevisiae* is compared.

2. Materials and Methods

2.1. Microorganisms and Culture Conditions

A thermotolerant yeast, *Kluyveromyces* sp. IIPE453 (MTCC 5314) [19] was grown in Bioflow-110 bioreactor (working volume 5 liters) on medium SM containing g l^{-1}, di-sodium hydrogen ortho phosphate, 0.15; potassium di-hydrogen ortho phosphate, 0.15; ammonium sulphate, 2.0; yeast extract, 1.0; glucose 20. The temperature and pH were controlled at 50 ℃ and 5.0, respectively. The aeration rate in the bioreactor was provided at 1vvm and the agitation was maintained at 350rpm.2.1.1. Front Matter

2.2. Fermentation Conditions

The batch fermentation using the yeast Kluyveromyces sp. IIPE453 was carried out in a Bioflow-110 bioreactor (working volume 2 liters) using molasses as substrate, which contained the initial reducing sugar concentration of 300g l^{-1} [20]. The components (in g l^{-1}), di-sodium hydrogen ortho phosphate, 0.15; potassium di-hydrogen ortho phosphate, 0.15; ammonium sulphate, 1.0; yeast extract, 1.0 were mixed in the molasses solution and sterilized for 15min at 121 ℃. The temperature and pH were controlled at 50 ℃ and 5.0, respectively. The agitation was maintained at 200 rpm. The initial dry cell weight (DCW) was kept at 4.5g l^{-1}.

2.3. Bioreactor

Bioflow-110 bioreactor of working volume 2 liters was used in the study. The bioreactor vessel was made of glass with internal diameter of 12.8cm, height of broth in the reactor of 17cm and the thickness of glass of 0.3cm, as shown in Figure 1.

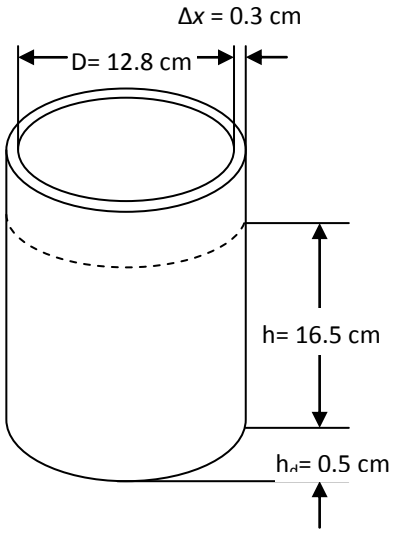

Figure 1. The illustration of bioreactor vessel

3. Theory/Calculation

3.1. Heat Generation

The ethanol fermentation process is an exothermic process. As the fermentation progresses (glucose fermentation to ethanol proceeds), the heat generated will raise the temperature of the fermentation broth. To maintain isothermal conditions, the excess heat generated is removed by using cooling coils within the reactor and/or jackets around the reactor with continuous flow of cooling medium (cold/chilled water). In a laboratory, with no fan/ventilator, the heat loss from the bioreactor surface to the surrounding air atmosphere shall take place by natural convection. If the reactor is used for cell growth, then aeration is done at a rate suitable for maintaining growth. In such a case, heat loss to the surrounding shall take place by (i) convective heat transport from the broth by bubbling air, (ii) natural convection from the reactor surface to the surrounding atmosphere, and (iii) heat loss by evaporation of water and ethanol.

The heat generation during growth or fermentation in a batch reactor can be given as:

$$Q = mC_p\Delta T + Q_{loss} \tag{2}$$

Where, Q is the heat generated (kJ); m is the mass of fermentation/growth medium solution (kg); C_p is the specific heat (kJ kg^{-1} K^{-1}); ΔT is the temperature difference of the fermentation broth over a time period (Δt) (K); and Q_{loss} is the total heat loss from the reactor accounting for the heat loss due to aeration (during cell growth operation) and the heat loss from the reactor surface by natural convection. On differentiating Eq. (2) with respect to time, one gets

$$\dot{Q} = \frac{dQ}{dt} = mC_p \left.\frac{dT}{dt}\right|_{with\ active\ cells} + \dot{Q}_{loss} \tag{3}$$

Therefore,

$$\dot{Q} - \dot{Q}_{loss} = mC_p \left.\frac{dT}{dt}\right|_{with\ active\ cells} \tag{4}$$

Where, $\dot{Q}$ is the heat generated per unit time (kJ h^{-1}); C_p is considered to be insensitive to temperature change over a small temperature difference ($\Delta T \approx 3$ K); dT/dt is the slope of the curve between temperature and time, over a given time interval.

With inactive cells in the reactor, the temperature of the reactor will fall with time due to heat loss from the reactor surface to the surrounding.

Thus,

$$\dot{Q}_{loss} = mC_p \left.\frac{dT}{dt}\right|_{with\ inactive\ cells} \tag{5}$$

Therefore, one gets,

$$\dot{Q} = mC_p \left.\frac{dT}{dt}\right| \tag{6}$$

3.2. Heat Loss

The heat losses in a bioreactor during operation at high temperature may take place due to (i) convective heat transport from the broth by bubbling air, (ii) natural convection from the reactor surface to the surrounding atmosphere, and (iii) heat loss by evaporation of water and ethanol. The heat losses due to radiation, conduction and CO_2 are assumed to be negligible.

The heat loss due to bubbling air and evaporation can be given as:

$$\dot{Q}_a = mC_p \left.\frac{dT}{dt}\right|_{\text{with inactive cells \& without aeration}} - mC_p \left.\frac{dT}{dt}\right|_{\text{with inactive cells \& aeration}} \quad (7)$$

Where, $\dot{Q}_a$ is the heat loss due to bubbling air and evaporation (kJ h^{-1}).

The heat loss due to natural convection can be given as [21]:

$$\dot{Q}_c = UA\Delta T \quad (8)$$

Where, $\dot{Q}_c$ is the heat loss due to natural convection (kJ h^{-1}); U is the overall heat transfer coefficient (kJ h^{-1} m^{-2} K^{-1}); A is the surface area (m^2) and ΔT is the temperature difference between the reactor surface and the surrounding atmosphere (K). The overall heat transfer coefficient can be given as:

$$U = \frac{1}{\frac{1}{h_b} + \frac{\Delta x}{\lambda} + \frac{1}{h_a}} \quad (9)$$

Where, h_b and h_a are the convection heat transfer coefficients for broth and air, respectively (kJ h^{-1} m^{-2} K^{-1}); Δx is the thickness of wall (m) and λ is the thermal conductivity of material (kJ h^{-1} m^{-1} K^{-1}).

For the natural convection, the convection coefficient (h_a) can be given as:

$$h_a = \frac{Nu\lambda_a}{H} \quad (10)$$

Where, *Nu* is the Nusselt number; λa is the thermal conductivity of the air (kJ h^{-1} m^{-1} K^{-1}); and H is the height of the broth level in bioreactor (m). The Nusselt number can be evaluated as reported by Lienhard and Leinhard [22]:

$$Nu = 0.678Ra^{1/4}\left(\frac{\Pr}{0.952+\Pr}\right)^{1/4} \quad (11)$$

Where, *Ra* is the Rayleigh number; and *Pr* is the Prandtl number which can be given as:

$$Ra = \frac{g\left(\frac{1}{T_a}\right)(T_b - T_a)H^3}{\nu_a \alpha_a} \quad (12)$$

and,

$$\Pr = \frac{\nu_a}{\alpha_a} \quad (13)$$

Where, g is the gravity constant (m s^{-2}); T_b and T_a are the temperatures of broth and surrounding atmosphere, respectively (K); va and αa are the kinametic viscosity and thermal diffusivity of air (m^2 s^{-1}).

Surface area (*A*) of bioreactor can be given as:

$$A = 2\pi rh + 2\pi r_d h_d \quad (14)$$

Where, *r* and *h* are the radius and height of the level of broth in cylindrical section, respectively (m); r_d and h_d are the radius and height of spherical bottom, respectively (m). r_d can be calculated as:

$$r_d = \frac{4h_d^2 + D^2}{8h_d} \quad (15)$$

4. Results and Discussion

The temperature-time courses were recorded over a period of 20min in lag phase (0 hour) and log phase (12th hour) during the growth of yeast Kluyveromyces sp. IIPE453 at 50°C at 1 vvm aeration rate (Figure 2). The temperature was not controlled during recording of the temperature-time course. As shown in Figure 2, the temperature was decreased with time during lag phase because of high rate of heat loss to surrounding atmosphere. The lower rate of heat loss was observed during log phase than that of lag phase, shows the heat generation due to the growth of yeast. The heat generation due to growth of yeast was calculated using Eq. (6), which was found to be 26.1kJ h^{-1}. The sugar consumption rate in 12th hour of the growth of yeast was found to be 0.04mol h^{-1}. Therefore, the heat generation will be 652kJ mol^{-1} of sugar consumed during the growth of yeast *Kluyveromyces* sp. IIPE453 at 50 ℃.

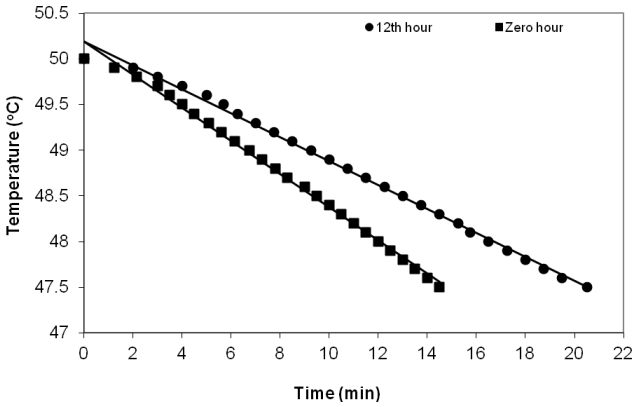

Figure 2. Temperature-time course of bioreactor during growth of thermotolerant yeast *Kluyveromyces* sp. IIPE453 at 50 ℃ with 1 vvm aeration

The temperature-time courses were recorded over a period of 15min between zero and 24th hour during a batch fermentation using yeast *Kluyveromyces* sp. IIPE453 at 50 ℃. The temperature was not controlled during recording of the temperature-time course. As shown in Figure 3, the temperature was decreased with time because of high rate of heat loss to surrounding atmosphere at zero hour. The rate of heat loss was lower at 12th hour than that of zero hour, shows the heat generation due to the ethanol fermentation. The heat generation was calculated using Eq. (6), as given in Table 1. Figure 4 shows the heat generation in corresponding to sugar consumption rate and ethanol production rate. The heat generated in ethanol fermentation using *Kluyveromyces* sp. IIPE453 was found to be 132.54kJ mol^{-1} of sugar consumed or 67.84kJ mol^{-1} (2.46MJ l^{-1}) of ethanol

produced at 50 ℃. Theerarattananoon et al. [16] reported heat generation of 43-66kJ mol^{-1} of sugar consumed by *S. cerevisiae* at 25 ℃, which is almost half of the heat generated during ethanol fermentation using *Kluyveromyces* sp. IIPE453.

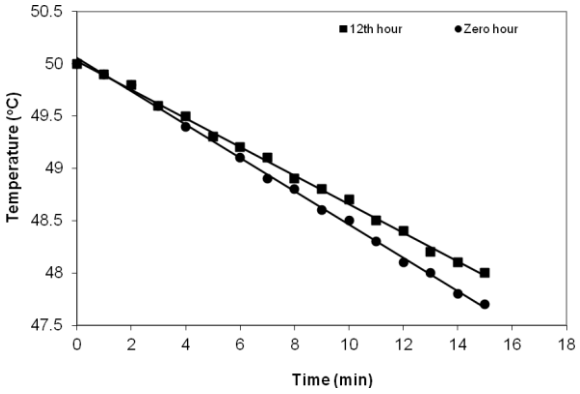

Figure 3. Temperature-time course of bioreactor during ethanol fermentation using thermotolerant yeast *Kluyveromyces* sp. IIPE453 at 50 ℃

Table 1. Variation in rate of heat generation with rates of sugar consumption and ethanol production at different times during ethanol fermentation using *Kluyveromyces* sp. IIPE453 at 50 ℃

Time (h)	Sugar consumption rate (mol h^{-1})	Ethanol production rate (mol h^{-1})	Heat generated (kJ h^{-1})
4	0.09	0.18	12.6
8	0.125	0.24	17.2
12	0.21	0.41	27.4
20	0.17	0.34	23.0
24	0.15	0.30	20.1

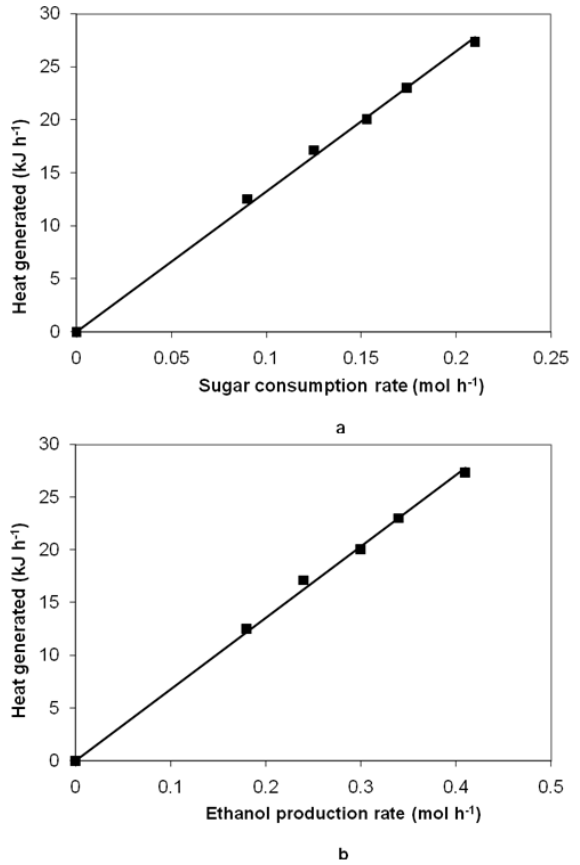

Figure 4. Heat generated during ethanol fermentation using thermotolerant yeast *Kluyveromyces* sp. IIPE453 at 50 ℃ at different (a) sugar consumption rates and (b) ethanol production rates

The heat loss due to bubbling air (1 vvm aeration rate) and evaporation was calculated using Eq. (7), which was found to be 36kJ h^{-1}. The surface area of the bioreactor vessel was calculated using Eq. (14) as 0.079m^2. The heat loss due to natural convection was calculated using Eqs. (8)-(13) and found to be 187kJ h^{-1}. The kinematic viscosity (ν_a), thermal diffusivity (α_a) and thermal conductivity (λ_a) of air at surrounding temperature of 25°C are used as 1.595x$10^{-5}$$m^2$ s^{-1}, 2.371x$10^{-5}$$m^2$ s^{-1} and 9.36kJ h^{-1} m^{-1} K^{-1}, respectively. The convection heat transfer coefficient (h_a), thermal conductivity of glass material (λ) and thickness of wall (Δx) are used as 108kJ h^{-1} m^{-2} K^{-1}, 4.32kJ h^{-1} m^{-1} K^{-1} and 0.003m, respectively. In actual practice, the heat loss due to natural convection was calculated from Figure 2, which was found to be 58.7kJ h^{-1}

In case of growth, the total heat loss will be the sum of heat loss due to bubbling air, natural convection and evaporation, which was found to be 94.7kJ h^{-1}. For a growth of *Kluyveromyces* sp. IIPE453, a run of 24h [19], the total heat loss will be 2272.8kJ. The metabolic heat generation for 40g (0.222mol) sugar will be 145kJ. There is huge difference between heat loss and heat generation. The heat loss due to natural convection can be minimized using insulation and/or by decreasing S/V ratio. But heat loss due to bubbling air and evaporation cannot be controlled unless air at high temperature introduced in the reactor. The heat loss due to bubbling air and evaporation in 24h will be 864kJ. Therefore, during growth of the yeast the heat input will be required to maintain the desired temperature in the bioreactor.

In case of fermentation, the total heat loss will be due to natural convection, which is found to be 58.7kJ h^{-1}. For a run of ethanol fermentation on molasses in first 32h, the total sugar consumption was 334g or 1.86mol [20]. The total heat loss in 32h will be 1878.4kJ. The metabolic heat generation for 1.86mol sugar will be 212kJ. There is also a huge difference between heat loss and heat generation. The heat loss due to natural convection can be minimized using insulation and/or by decreasing S/V ratio. Therefore, no additional heat would be required to maintain the desired temperature for fermentation in the bioreactor.

In a case study of Indian distillery, fermentation of 400 m^3 wort using *S. cerevisiae* completes in 32h with 8% (v/v) ethanol concentration, which requires cooling water at 25 ℃ at the rate of 550m^3 h^{-1}. Therefore, 550m^3 cooling water per m^3 of ethanol is required to maintain temperature at 32 ℃ in a bioreactor. The power requirement for cooling tower and circulating to the bioreactor is ~70Kw h^{-1} or ~0.07kW l^{-1} of ethanol produced. For a fermentation run, the cooling water is required for 20h, which will consume ~5.04MJ l^{-1} of ethanol produced. The energy required for fermentation and distillation is 12.63MJ l^{-1} of ethanol [23]. Therefore, about 40% of energy utilization can be minimized by eliminating the cooling during ethanol fermentation. The metabolic heat may be sufficient to maintain the temperature of bioreactor at 50 ℃.

5. Conclusions

The metabolic heat generated due to ethanol fermentation by thermotolerant yeast *Kluyveromyces* sp.

IIPE453 was higher than reported for mesophilic yeast *S. cerevisiae*. Ethanol fermentation using thermotolerant yeast has the advantage over mesophiles in terms of minimization of the cooling water requirement. Therefore, no additional heat may be required to maintain the temperature in fermentation process by thermotolerant yeast at 50 ℃. In addition, using thermotolerant yeast for ethanol fermentation power requirement for maintaining temperature in a bioreactor can be minimized, which accounts ~40% of total power requirement in fermentation and distillation using mesophiles. The current study is a lab-scale study and future study on a large-scale is required to validate the results.

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Nomenclature

A Surface area (m^2)
C_p Specific heat (kJ kg^{-1} K^{-1})
D Diameter of cylindrical section of bioreactor (m)
dT/dt Slope of the curve between temperature and time
g Gravity constant (m s^{-2})
h Height of the broth level in bioreactor (m)
h Height of the level of broth in cylindrical section (m)
h_a Convection heat transfer coefficients for air (kJ h^{-1} m^{-2} K^{-1})
h_b Convection heat transfer coefficients for broth (kJ h^{-1} m^{-2} K^{-1})
h_d Height of the bottom of bioreactor (m)
ΔHr Heat generated due to biological reactions (kJ)
M Mass of fermentation/growth medium solution (kg)
Nu Nusselt number
Pr Prandtl number
Q Heat generated (kJ)
$\dot{Q}$ Heat generated per unit time (kJ h^{-1})
Q_{loss} Total heat loss from the reactor (kJ)
$\dot{Q}_a$ Heat loss per unit time due bubbling air and evaporation (kJ h^{-1})
$\dot{Q}_c$ Heat loss per unit time due natural convection (kJ h^{-1})
R Radius of cylindrical section of bioreactor (m)
r_d Radius of sphere of the bottom of bioreactor (m)
Ra Rayleigh number
T_a Temperature of surrounding atmosphere (K)
T_b Temperature of broth (K)
ΔT Temperature difference between the reactor surface and the surrounding atmosphere (K)
U Overall heat transfer coefficient (kJ h^{-1} m^{-2} K^{-1})
Δx Thickness of wall (m)

Symbols

Λ Thermal conductivity of material (kJ h^{-1} m^{-1} K^{-1})
λ_a Thermal conductivity of the air (kJ h^{-1} m^{-1} K^{-1})
ν_a Kinametic viscosity of air (m^2 s^{-1})
α_a Thermal diffusivity of air (m^2 s^{-1})

Subscripts

A Air
B Broth
C Natural convection
D Bottom of bioreactor

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