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# Application of hydrocyclone for removal of yeasts from alcohol fermentations broth

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**Abstract**

In this research work, the possibility of using hydrocyclones to separate *Saccharomyces serevisiae* 5209 from a prepared media was studied. The cell separation can be very useful in order to improve the quality of alcohol produced, reduce energy consumption and also reduce the cost of effluent treatments with the hydrocyclone advantage of consuming less energy. A hydrocyclone with 15 mm internal diameter was used for cell separations. A better separation efficiency of 3.84 (gram cell dry weight in: under flow/over flow) was obtained at volumetric flow rate of about 112 $cm^3/s$.


*Keywords:* Hydrocyclones; Ethanol fermentation; Separation; Centrifugation

## 1. Introduction

Hydrocyclones are very important for their use in separation of liquid–liquid, solid–liquid and gas–liquid in large scales. Recent applications of hydrocyclones for separation of microbial cells and mammalian cells are promising by cutting down the dimensions sizes [1,2]. The findings may be useful in applying the concepts for separation of proteins and large biomolecules in future.

Centrifuges and hydrocyclones are the two means by which clarification of fruit juices, crystal separation, cell separations, effluent treatment and thickening in industries is achieved [3]. The principles of separation behind centrifuges are acceleration. Svedburg (Swedish biochemist, 1923) applied enormous accelerations and attained rotational speeds as high as 80,000 rpm to generate centrifugal fields in excess of 600,000 × $g$. This is the principal idea behind ultracentrifuge technology now applied in proteins, DNA, RNAs as well as biomolecules separation.

Hydrocyclones consists of two main parts (Fig. 1). A cylindrical part, with an inlet through which the feed enters tangentially. In this part an outlet is located vertically on top of cylinder which extends within the cylinder and called vortex finder [1]. The second main part is conical part connected to the cylindrical section on the top and to the underflow from bottom end is called spigot. The centrifugation forces exerted by vortex carry larger particles to the cyclone wall; these are discharged by underflow orifice. Small particles moved to the central axis of the cyclone and carried out by the overflow stream [2].

Although design and manufacture of industrial hydrocyclones is relied more on experience. Their performance has been documented by geometries, most notably cone angles and orifice sizes. The effect of these geometries is generally expressed as a function of changes in the expected cut point or $D_{50}$ that the hydrocyclone will provide [4]. In recent years, experimental works [5] and the computational fluid dynamics (CFD) and modeling technology has revolutionized the method in which fluid dynamics is practiced and in the case of hydrocyclones alone has become a preferred development tool [6,7].

Hydrocyclones have if at all a very low footprint in our food and biotechnological industries and even in effluent treatments. This lack of attention could be simply overcome if one just considers the advantages: suitability for continuous operations, high efficiency, minimal space and low maintenance requirements, easy and rapid in particles separation, save energy, low cost and particles size recovery in wider range. It is also very important to note that there is no disruption of cells in the process of separation [8]. A very important and yet necessary application

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**Nomenclature**

| | |
|---|---|
| $c$ | Solids concentration of feed suspension |
| $C$ | Concentration ratio |
| $d_p$ | Particle diameter |
| $D_c$ | Cyclone diameter |
| $D_i$ | Inlet diameter |
| $k$ | Constant |
| $L$ | Length of cyclone |
| $\Delta P$ | Operation pressure |
| $\Delta P_s$ | Static pressure drop |
| $Q$ | Volumetric flow rate in feed |
| $Q_u$ | Volumetric flow rate in underflow |
| $r$ | Radial position |
| $R$ | Fractional recovery of solids to underflow |
| $R_v$ | Fractional volumetric recovery of suspension to the underflow |
| $t$ | Time |
| $T$ | Temperature |
| $U_r$ | Particle terminal velocity in radial direction |
| $v_i$ | Inlet fluid velocity |
| $v_t$ | Fluid velocity in tangential direction |
| $v_z$ | Fluid velocity in axial direction |
| $z$ | Axial position |

*Greek letters*

| | |
|---|---|
| $\rho$ | Density of suspending medium |
| $\rho_s$ | Density of solid particle |
| $\mu$ | Viscosity of suspension |
| $\mu_s$ | Viscosity of the suspending medium |
| $\tau$ | Residence time |

of hydrocyclones is alcohol fermentation were a cell concentration of ∼5 g is used for ethanol production in one litter of broth. *Saccharomyces serevisiae*, a unicellular microorganism is used as the usual yeast in fermentation industries. In separation of alcohol from fermentation broth the cells are not usually removed. The disadvantages of not removing the cells and residues are production of low grade ethanol, dead flux, higher maintenance cost and save energy on pumps with further difficulties in effluent treatment which by itself causes a COD content of ∼2–5000 mg/l.

Viscosity of fermentation broth usually affects the separation efficiency. An equation relating viscosity as a function of biomass developed [9]. However, cell suspension viscosity is also affected by temperature. Therefore, a relationship between suspension viscosity based on cell concentration and temperature is required. The later relationship is used to determine the dependency of the separation efficiency on the viscosity of the suspension [10].

As any other processes, the separation in a hydrocyclone is not complete. A fraction of the water in the feed reports to the concentrated underflow and also, a fraction of cells are reported to the vortex finder. For quantification of overall separation performance two values are required; $R$, the recovery of cells from the feed to the underflow, and $C$, the ratio of the concentrations of the underflow to the feed. Therefore, the volumetric recovery of suspension to the underflow, $R_v$ could be expressed as the ratio of $R$ to $C$.

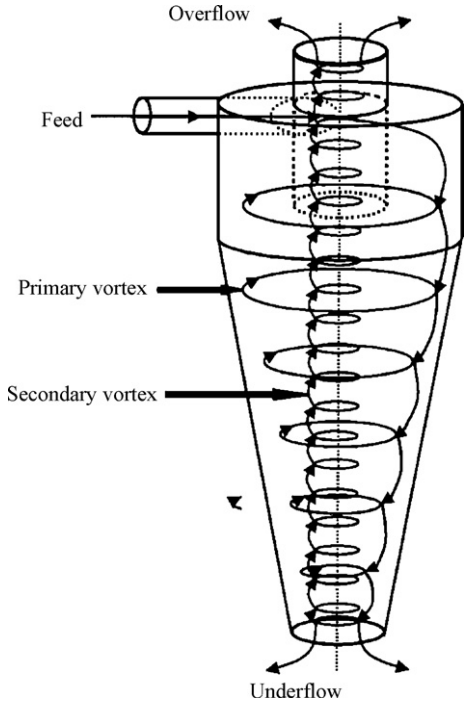


Fig. 1. Schematic of a hydrocyclone.

This paper presents experiments in order to find out the effect of cell concentration (i.e. yeasts from alcohol fermentation broth) as well as volumetric flow rates on a mini-hydrocyclone.

## 2. Materials and methods

### 2.1. Organism and culture maintenance

*Saccharomyces sereviseae* IROST 5209 obtained from Iranian Research Organization for Science and Technology (IROST), Tehran maintained as slant on Potato-Dextrose-Agar (PDA) for 5 days before use. The organism was subcultured at regular intervals of 15 days and stored at $4 \pm 1\,^{\circ}C$.

### 2.2. Culture medium and culture conditions

Yeast from 5-day slant suspended in 10 ml sterile water. Concentration of yeasts in suspension was measured by counting spores using a hemocytometer. A volume of this suspension containing $8 \times 10^6$ cells added to 100 ml medium. The inoculum culture media were cultivated in 500 ml shake flasks, with 100 ml media consisted (g/l): beet molasses 80; $NaNO_3$ 1.0; $NaH_2PO_4$ 3.0, $MgSO_4{\cdot}7H_2O$ 0.75 and initial pH of 4.5. The culture media was autoclaved and after cooling to room temperature left on a rotary shaker at 160 rpm and 30 °C for 3 days for complete growth. The inoculums after growth were centrifuged under aseptic condition (at $4300 \times g$ for 10 min).

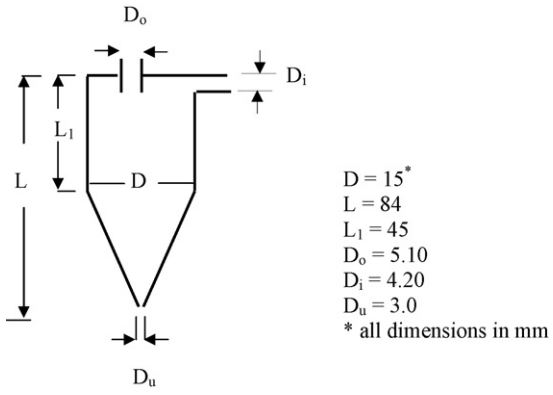


Fig. 2. Schematic dimension of hydro cyclone in study.

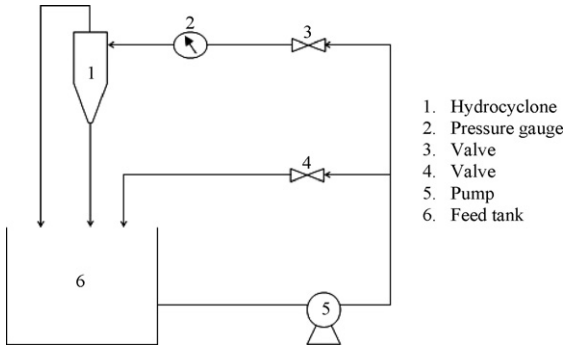


Fig. 3. Experimental set up.

### 2.3. Ethanol production media

Ethanol production media were prepared by adding g/l: 350 beet molasses, 1.74 urea, 3 phosphate fertilizer and a cell mass of 5 g/l and initial pH of 5.5 adjusted. A 1.5 l of this media in 3 l Erlenmayer flasks under aseptic conditions left on a rotary shaker at 160 rpm and 30 °C for 2 h for adaptation. The content added to a sterile cylindrical glass with working volume of 20 l. After no self-agitation of medium (hence no $CO_2$ and no more alcohol production) the medium was left for cell settling. The supernatant was removed and heated to 90 °C for 2 h for ethanol removal. The content was diluted to obtain different cell concentrations as required by experimental plan.

### 2.4. Hydrocyclone dimensions

Hydrocyclones used in this study was small (Fig. 2) with following dimensions all in mm: inlet orifice ($D_i$) 4.20; over flow orifice ($D_0$) 5.10; under flow orifice ($D_u$) 3.00; total length 84.00; cylindrical length ($L_1$) 45.00.

### 2.5. Experimental part

The remaining yeast cells after settling, and heating then diluted in to three different initial concentrations, g/l: 0.036, 0.078 and 0.13. At each initial cells concentration three different volumetric flow rates were tested. Due to practical reasons, it was difficult to fix the volumetric flow rate for all initial cells concentration alike, but read from the gauge directly. Initial cells concentration, initial volumetric flow rate and the results obtained are shown in Table 1. Cells concentration were counted using hemocytometer for inlet, under flow and over flow. The results expressed in cells dry weight using the relation $1\,g = 3.06886 \times 10^{10}$ cells. The experimental set up is shown in Fig. 3.

## 3. Theories of separation

The velocity of flow in hydrocyclone can be resolved into three components, tangential, axial and radial. A particle within a hydrocyclone will balance itself between the increasing centrifugal velocity and a decreasing radial fluid velocity towards the center of hydrocyclone. Several mathematical description based on theory are expressed by modeling hydrocyclone performance [3]. The proposed crowding theory [11] suggested that the cut size is the function of capacity of underflow and particle size distribution of the feed. Similarly later a separation model based on turbulence two phase flows was proposed [12]. The equilibrium orbit theory takes no account of residence time [13]. The non-equilibrium theory (residence time theory) which considers the particle if entering precisely in the center of the inlet pipe will just

Table 1
Experimental results obtained with hydrocyclone

| Volumetric flow rate ($cm^3/s$) | | | Cells concentration (g/l) | | | Cells ratio (under flow/over flow) | Efficiency |
|---|---|---|---|---|---|---|---|
| Inlet | Under flow | Over flow | Inlet | Under flow | Over flow | | |
| 57.895 | 20 | 37.895 | 0.036 | 0.010 | 0.026 | 0.4 | 27.8 |
| 72.718 | 17.53 | 55.188 | | 0.021 | 0.016 | 1.33 | 58.3 |
| 91.108 | 19.223 | 71.885 | | 0.027 | 0.009 | 2.87 | 75 |
| 112.495 | 21.655 | 90.84 | | 0.028 | 0.008 | 3.84 | 77.8 |
| 68.599 | 13.408 | 55.191 | 0.078 | 0.039 | 0.039 | 1 | 50 |
| 87.084 | 15.038 | 72.046 | | 0.053 | 0.025 | 2.12 | 67.9 |
| 95.982 | 16.04 | 79.942 | | 0.055 | 0.023 | 2.33 | 70.5 |
| 112.78 | 15.839 | 102.941 | | 0.056 | 0.022 | 2.58 | 71.8 |
| 62.816 | 15.727 | 47.089 | 0.13 | 0.064 | 0.066 | 0.96 | 49.2 |
| 84.203 | 17.536 | 66.667 | | 0.065 | 0.065 | 1 | 50 |
| 94.758 | 15.413 | 79.345 | | 0.085 | 0.046 | 1.85 | 65.4 |
| 112.982 | 12.982 | 100 | | 0.090 | 0.040 | 2.23 | 69.23 |

rich the wall in residence time $\tau$ [14]. Cilliers and Harrison [15] applied the residence time theory and derived an equation for recovery for microorganisms using mini-hydrocyclone. In our experiments too, this theory is the most suitable for theoretical explanation of experimental results. Therefore, as described for a hydrocyclone with a diameter $D_i$ a particle of size $d_p$ in a centrifugal field settles at its terminal velocity in a radial direction $U_r$, and fluid density $\rho$ and within a fluid viscosity, $\mu$ according to:

$$u_r = \frac{(\rho_s - \rho) d_p^2 v_r^2}{10\,\mu r} \tag{1}$$

The fraction of recovery of particles to the underflow, $R$, for a monosized feed can be described by:

$$R = \frac{\int_0^T u_r dt}{D_i} \tag{2}$$

The centrifugal head and the static pressure drop are related as follow:

$$\Delta p_s = \int_0^{D_c/2} \rho \frac{v_t^2}{r} dr \tag{3}$$

The axial velocity is given as:

$$v_z = \frac{dz}{dt} \tag{4}$$

It is assumed that the flow near the wall follows the wall contour:

$$\frac{dz}{dr} = 2\frac{L}{D_c} \tag{5}$$

Combining Eqs. (1)–(5), the recovery of particles to underflow, $R$, could be expressed as:

$$R = \frac{d_p^2(\rho_s - \rho)}{9\rho D_i} \frac{L}{D_c} \frac{\Delta P_s}{v_z} \frac{1}{\mu} \tag{6}$$

The volumetric throughput, $Q$, of the hydrocyclone for a given geometry is related to the total pressure drop, $\Delta P$, by:

$$Q = k\Delta P^{0.5} \tag{7}$$

This is in agreement with the theoretical prediction of flow through a pipe [14] therefore;

$$Q = \frac{\pi}{4} D_i^2 v_i \tag{8}$$

The difference between the static and total pressure drop for efficient hydrocyclone is small. Therefore, combining Eqs. (6)–(8), the recovery can be written as:

$$R = \frac{k d_p^2(\rho_s - \rho)}{36\rho} \frac{L\pi D_i v_i}{D_c v_z} \Delta P^{0.5} \frac{1}{\mu} \tag{9}$$

For a given hydrocyclone geometry the ratio of the inlet to axial velocities at high Reynolds numbers can be assumed to be constant and Eq. (9) can be reduced to:

$$R = k_{particle} k_{geometry} \Delta P^{0.5} \frac{1}{\mu} \tag{10}$$

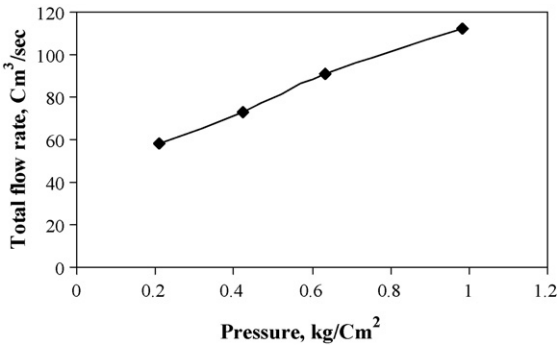


Fig. 4. The relationship between the total feed flow rate and pressure drop across the hydrocyclone.

This indicates that, the recovery is inversely proportional to viscosity and directly proportional to the pressure drop for a given particle and constant geometry of the hydrocyclone.

## 4. Results and discussion

The separation efficiency of a hydrocyclone in order to recover the higher concentration of microbial cells will certainly depends on many factors such as pressure drop, flow rate, particle size and geometry of the hydrocyclone. At a fixed geometry the flow rate and feed cell concentration will be the only factors which has maximum effect on recovery. An increase in cell concentration will affect the viscosity of the flow and this increase has a negative effect on recovery of cells by hydrocyclone. It is to notice that always separation in the hydrocyclone is not completed because the partial feed concentration will be present at under flow rate and partially at overflow. It is expected that small cell size will be present in overflow and large ones in underflow. In order to quantify the performance of a hydrocyclone we will be requiring two values, one is the solid recovery of the feed to the under flow, $R$, and the concentration ratio, $C$. The ratio of the concentration of the underflow to the overflow has to be maximized in order to attain maximum recovery.

Table 1 shows the experimental as well as efficiency of the hydrocyclone in separating the cells. As mentioned in Section 2 three initial cell concentrations were studied each with four different volumetric flow rates. For all initial cell concentrations under study, recovery was increased by increasing the flow rate but in the first part of experiment with cell concentrations of $0.036\,g\,l^{-1}$ it is noticed that due to small sizes of the cells (i.e.

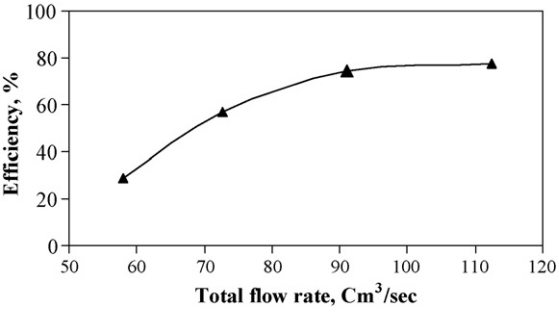


Fig. 5. The effect of flow rate on recovery.

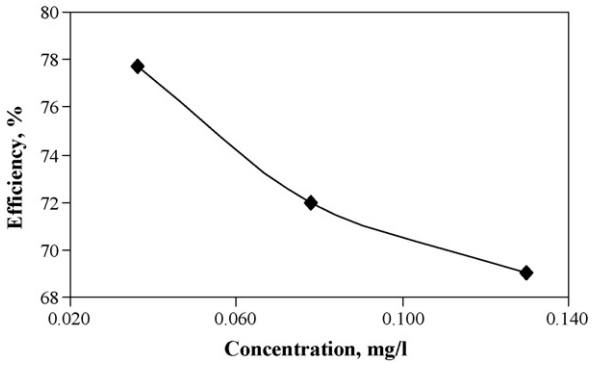


Fig. 6. Effect of feed concentration on recovery.

∼5–10 μm) higher flow rates are more favorable. In addition to this the ratio of cells collected at under flow discharge to the feed concentration was very noticeable that is maximum 3.84 at a flow rate of 112.49 $cm^3/s$.

The relationship between the flow rate and the pressure drop is shown in Fig. 4. The experimental results indicate that an increase in flow rate will have a positive effect on pressure drop. This is also noticed by Eq. (7).

Fig. 5 shows that for a given geometry of hydrocyclone an increase in flow rate will increase the recovery, this was obtained by treating a yeast suspension of 0.036 $gl^{-1}$. Since increasing the flow rate will affect the pressure drop within the hydrocyclone thereby recovery will increase as can be easily explained based on Eq. (10).

By increasing the concentration of microbial cells in the feed, the recovery of cells by hydrocyclone will be reduced. This is clear from Fig. 6. Moreover, the increase of cell concentration will increase the viscosity of broth, thereby decreasing the recovery according to Eq. (10). It is important to clarify this point that even though more feed cell concentration seems to be important in handling and separating of more microorganisms in industry but, the recovery will be reduced. Therefore, it is better to use more number of hydrocyclones with low feed cell concentrations rather than a single hydrocyclone with high feed concentration.

## 5. Conclusion

It was shown that separation of microbial cells could be employed successfully using a 15 mm hydrocyclone. Cell recovery is a function of feed concentration and flow rate, more over there was no disruption of cells in underflow as well as overflow. The recovery of cells to the under flow and the concentration ratio attained are a function of the operating pressure and feed concentration of suspension. It is possible to improve the cell recovery by further geometrical consideration. Acceptable recovery levels and concentration ratios is favored when treating low feed concentrations. Using multi-hydrocyclone will allow higher volume of feed for treatment.

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