Oxygen transfer and dimethylketone consumption rate in the airlift reactor using bacteria immobilized in an inverse fluidized bed
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**Oxygen transfer and dimethylketone consumption rate in the airlift reactor using bacteria immobilized in an inverse fluidized bed**

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**Abstract**

Immobilized *Acinetobacter calcoaceticus* bacteria are used for the biodegradation of the dimethylketone in the inverse fluidized bed airlift reactor. The expanded polystyrene particles are used as the bacteria carrier. A diminishing of the minimum fluidization velocity is observed during the biodegradation time. A lowest degradation time is observed if the gas velocity ranges between the minimum fluidization velocity and the approximately double value of the minimum fluidization velocity. The biodegradation time decreases with an increase of the initial bed height with the immobilized bacteria i.e. with an increase of the contact time between the bacteria and the dimethylketone solution. The effective oxygen accumulation rate decreases with an increase of the bed height with immobilized bacteria.

Keywords: waste water, dimethylketone, biodegradation time, oxygen accumulation rate

**1. Introduction**

Degradation of the anthropogenic compounds depends on their chemical structure. Alkanes, alcohols as well as aromatic and cyclic hydrocarbons are successfuslly biodegraded using *Acinetobacter calcoaceticus* bacteria [1-6]. It is well known that the cell sensitivity to the mechanical action is the main restriction for the scale-up of the cell culture. An agitation and cell collisions are main factors causing the cell destruction. The cell sensitivity is a result of the rigid wall lack. The immobilization of the microorganism cells is one of the method of prevention from the destruction of the physical cell structure.

Taking into account, that the alive microorganisms are sensitive to shear stresses, the airlift bioreactor seems to be the more convenient reactor type than the stirred bioreactor [7]. A location of microorganisms is reasonable in the downcomer of the airlift reactor because shear forces are significantly lower there than these in the riser [8, 9]. The solid density have to be lower than the liquid density to create the inverse fluidized bed [10 – 12] in the downcomer because of the existing liquid downflow.
Oxygen is absorbed in the aerated riser and delivered in the circulated liquid stream for bacteria placed in the downcomer. Immobilization of microorganisms in the biofilm covering the solid particle promotes the microorganism growth. Fluidization guarantees a growth of immobilized microorganisms in conditions of a good contact of the solid and liquid phases.

The aim of the work is to investigate the oxygen accumulation rate and the dimethylketone uptake rate in the inverse fluidized bed external loop airlift reactor if the *Acinetobacter calcoaceticus* bacteria are immobilized on solid particles creating the inverse fluidized bed in the downcomer.

### 2. Apparatus and procedure

Experiments are carried out in the external loop airlift reactor. The reactor consists of two vertical columns. The perforated teflon plate creating the riser bottom is the gas distributor with hole diameter of 0.5 mm. The riser and the downcomer diameters are $d_r / d_o = 0.051 / 0.060$ m and $D_r / D_o = 0.082 / 0.090$ m respectively. The reactor working volume is 9 dm$^3$. The liquid level is maintained at the height of 1.3 m above the bottom.

Polystyren spherical particles ($d_S = 2$ mm; $\rho_S = 200$ kg·m$^{-3}$) are used as the carrier of the *A. calcoaceticus* aerobacteria. Solid particles with immobilized bacteria are placed in the downcomer between two wire net baffles to avoid a particle circulation around the reactor loop. The top net baffle is mounted under the top riser – downcomer connecting tube. The lower net baffle is mounted above the bottom connecting tube.

The suspension of the *A. calcoaceticus* aerobacteria cells is placed in the bubble pre-column. 5 dm$^3$ of mineral salt solution and 5 cm$^3$ of dimethylketone are put in the column. Microorganisms were grown in the bubble pre-column during 24 h. After that time the polystyrene particles are placed in the bubble pre-column to immobilize bacteria. The immobilization is provided in the conditions of the nutrient deficit for 72 hours. The very low superficial air velocity is not higher than $2 \times 10^{-5}$ m·s$^{-1}$. After named time the polystyren particles with immobilized bacteria are filtrated and put into the downcomer of the airlift reactor.

The time-dependent concentration of the dimethylketone is measured using the gas chromatograph Perkin Elmer AutoSystem. Chromatograms are analysed using the computer program Turbochrom Professional.

The time-dependent oxygen concentration in the liquid is determined by the dynamic method [8, 13].

Liquid circulation velocity is determined by the tracer method [13, 14] by means of a pulse technique. A pulse of the aqueous saturated KCl solution is injected at the top of the downcomer. The pulse injection and the response signals are measured in the
same place. Electrodes cooperate with a conductometer and a recorder. The injected volume of the tracer is about onethousandth of the liquid volume in the reactor. Therefore the effect of the tracer amount upon the flow pattern is neglected. All experiments are repeated at least three times.

3. Substrate uptake rate and microorganism growth rate

The simplest model of the simultaneous substrate (dimethylketone) consumption and the biomass growth is described using two first order process rate equations:

$$ r = \frac{dc}{dt} = k_{de} \cdot c $$  \hspace{1cm} (1)

$$ r_X = \frac{dX}{dt} = \mu \cdot X $$  \hspace{1cm} (2)

where:

- $c$ - time-dependent dimethylketone (substrate) concentration, kg.m$^{-3}$
- $k_{de}$ - rate constant of the organic substance (substrate) uptake, h$^{-1}$
- $X$ - time-dependent biomass concentration, kg.m$^{-3}$
- $\mu$ - specific growth rate of the microorganism, h$^{-1}$

The degradation of the organic compounds in the solution and the synthesis of new cells occur simultaneously. The uptake rate of the substrate (dimethylketone) depends on the biomass amount and simultaneously the microorganism proliferation rate are dependent on the substrate concentration. It means that the $c$ and $X$ variables influence each other. Therefore the equation of the substrate uptake rate have to include a dynamics of the biomass growth.

$$ -\frac{dc}{dt} = \mu(c) \cdot X \cdot \frac{1}{Y} $$  \hspace{1cm} (3)

$$ Y = \frac{X - X_0}{c_0 - c} $$  \hspace{1cm} (4)

$$ \frac{dX}{dt} = \mu(c) \cdot X $$  \hspace{1cm} (5)

where:

- $c_0$ - initial concentration of the dimethylketone, kg.m$^{-3}$
- $c$ - concentration of the dimethylketone during the biodegradation time, kg.m$^{-3}$
- $\mu(c)$ - specific microorganisms growth rate dependent on the dimethylketone concentration
- $Y$ - biomass yield coefficient on substrate i.e. the ratio of the biomass growth rate to the dimethylketone consumption rate
The substrate consumption and the microorganism growth are investigated usually separately. Additionally the consumption rate of impurities during the aerobic wastewater treatment is measured most frequently without analysis of the microorganisms growth rate. Such calculations are also provided in the presented paper.

Integration of Eqn. (1) allows to obtain the following expression of the dimethylketone degradation constant:

\[ k_{de} = -\frac{1}{t} \ln \left( \frac{c}{c_0} \right) \]  

(6)

The final degradation time \( t_{degr} \) is found if the concentration of the dimethylketone decreased up to 5% of the initial dimethylketone concentration \( c_0 \).

The total contact time \( t_{cont} \) of the immobilized bacteria and the organic substance during the biodegradation solution can be calculated as follows:

\[ t_{cont} = N \cdot t_F = \frac{t_{de}}{t_c} \cdot t_F = t_{de} \frac{H}{t_c \cdot u_{LF}} \]  

(7)

Living cells influence the oxygen accumulation rate in the solution because they consume oxygen. The used dynamic method treatment of experimental points allows to find the time-dependent oxygen concentration in the bulk liquid. The measured oxygen concentration is a result of two processes i.e. the difference between oxygen transfer rate and the oxygen uptake rate by bacteria [15]

\[ \frac{dc}{dt} = k_{La} \left( c^* - c \right) - OUR = k_{La}^{app} \left( c^* - c \right) \]  

(8)

The oxygen accumulation rate coefficient \( k_{La}^{app} \) is calculated as usually in the coordinate system \( \ln \left| -\left( c / c^* \right) \right| = f(t) \) using least square method.

4. Results and discussion

Fig. 1 shows the course of the dimethylketone concentration during the degradation time for different pH values. It can be observed the shortest biodegradation time for pH value about seven. The degradation rate constant diminishes too if pH value decreases under seven (Fig. 2). It is worth to notice that the most intensive growth of the bacteria \( A. \ calcoaceticus \) for the named pH value [14] is very convenient from the practical point of view taking into account a possible application for the waste water treatment.
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Fig. 1. An influence of the pH on the degradation course of dimethylketone

\[ H_0 = 0.47 \text{ m}, \ c_0 = 0.2 \% \text{ vol}, \ u_{GRMF} = 0.045 \text{ m} \cdot \text{s}^{-1}. \]

Fig. 2. Dependence of the degradation constant on the pH of aqueous dimethylketone solution.

\[ H_0 = 0.4 \text{ m}, \ c_0 = 0.2 \% \text{ vol}, \ u_{GRMF} = 0.037 \text{ m} \cdot \text{s}^{-1}. \]
Fig. 3. Dimethylketone concentration changes during the degradation time in the inverse fluidized bed airlift reactor with immobilized bacteria for the gas velocity equal to the minimum fluidization velocity. $c_{b0} = 0.4\%$ obj. pH = 7

Figure 3 shows the dimethylketone concentration changes during the time for two bed heights i.e. 200 and 370. Presented experimental data for both cases are obtained for the gas velocity equal to the minimum fluidization velocity. Liquid stream is the fluidizing factor in the inverse fluidized bed airlift reactor. Minimum fluidization liquid velocity in the investigated reactor is described by the following experimental equation [13]:

$$Ar = \frac{734.34 \cdot Re_{LDMF} + 14.66 \cdot Re_{LDMF}^2}{10^{2.2}}$$

(10)

if the Archimedes number range is:

$$200 \leq Ar = \frac{d_s^3 \cdot (\rho_L - \rho_s) \cdot \rho_L \cdot g}{\eta_L^2} \leq 2 \cdot 10^6$$

(11)

It is interesting to notice that it was observed that the minimum fluidization velocity diminishes slightly during the degradation time. It is clear because the mean density of carrier particles increases during the degradation time as a result of an increase of the biofilm thickness.

It can be noticed (Table 1) that an increase of the bed height of solid particles covered by the immobilized bacteria *A. calcoaceticus* results in the increase of the degradation constant i.e. in the diminishing of the degradation time. Result is clear because the higher fixed bed means the larger number of immobilized bacteria as well as the larger value of the gas velocity. It is because the liquid circulation velocity at the onset of the fluidization is the same for each bed height, but the gas velocity at the onset of the fluidization is higher if the higher bed height is.
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Table 1. An influence of the initial bed height with the immobilized *A. calcoaceticus* bacteria on the degradation rate constant. pH = 7; \( c_0 = 0.2 \% \) vol.; \( u_{GR} = u_{GRMF} \).

<table>
<thead>
<tr>
<th>( H_0 ) [mm]</th>
<th>( k_{de} ) (Eqn.(6)) ( [h^{-1}] )</th>
<th>( R ) (Eqn. (6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.032</td>
<td>-0.993</td>
</tr>
<tr>
<td>300</td>
<td>0.051</td>
<td>-0.967</td>
</tr>
<tr>
<td>400</td>
<td>0.075</td>
<td>-0.982</td>
</tr>
<tr>
<td>500</td>
<td>0.109</td>
<td>-0.981</td>
</tr>
</tbody>
</table>

Besides an increase of the initial bed height means an increase of the cell culture amount in the bed. An increase of the bed height means also an increase of the contact time of dimethylketone with immobilized bacteria. Therefore an increase of the degradation constant is observed with an increase of the initial bed height (Table 1).

![Fig. 4. Dependence of the degradation constant on the air flow rate. pH = 7,0; \( c_0 = 0.2 \% \) obj., \( H_0 = 390 \) mm](image)

An influence of the gas velocity i.e. liquid circulation velocity on the degradation rate is very interesting (Fig. 4). The approximately constant lowest degradation rate constant is observed for the superficial air velocity ranging between minimum fluidization velocity \( u_{GRMF} = 0.037 \) m·s\(^{-1}\) and \( 0.073 \) i.e. about double \( u_{GRMF} \) value. The fixed bed exists for gas velocities lower than the velocity at the onset of the fluidization \( u_{GRMF} \). The distinct part of the biofilm covering the particle surface is not washed by the liquid in the fixed bed. The limited solid – liquid interface contact area is the reason of the slower biodegradation course in the fixed bed than in the fluidized bed.

An increase of the gas velocity above \( u_{GR} = 0.073 \) m·s\(^{-1}\) (Fig. 4) seems to be inconvenient. The reason could be the high liquid circulation velocity [16] resulting in the shear stress high enough for a destruction of bacteria cells. However it is well known that an increase of the gas velocity causes an increase of the oxygen transfer rate (Fig. 5). It means that the above described two contrary factors act. Therefore it
can be concluded that the most convenient gas flow velocity should be slightly higher than the minimum fluidization velocity.

![Graph showing influence of gas velocity on oxygen accumulation rate](image)

**Fig. 5.** Influence of the gas velocity on the oxygen accumulation rate in the fixed bed and in the inverse fluidized bed. \(d_S = 2.5 \text{ mm}, H_0 = 0.4 \text{ m}, \text{pH} = 7\)

In the investigated reactor oxygen is absorbed in the solution in the riser and is transported in the liquid stream into the downcomer. Immobilized bacteria in the downcomer need the oxygen as well as the organic substance for their metabolic processes. Biodegradation occurs in the biofilm covering fluidizing polystyren particles. Oxygen and organic substance diffuse from the liquid into biofilm and metabolism products diffuse into the liquid bulk.

The oxygen accumulation rate coefficient name is used in this paper however in the reactor without bacteria it is equal of course to the air – liquid oxygen transfer coefficient \(k_{La} = k_{La}^{app}\). Instead of this if the bacteria are present in the reactor they consume the oxygen. The applied dynamic method treatment of experimental points allows to obtain the oxygen concentration in the bulk liquid. Measured concentration values result from the difference between oxygen transfer rate and the oxygen uptake rate [14, 15] according to the equation

\[
\frac{dc}{dt} = k_{La}^{app} (c^* - c) - \text{OUR} = k_{La}^{ac} (c^* - c) \tag{9}
\]

An increase of the gas velocity causes an increase of the oxygen accumulation rate coefficient (Fig. 5). It can be observed that the coefficient for the system with bacteria is lower than the coefficient for the system without bacteria. Besides it is interesting to notice that the oxygen coefficient apparently decreases distinctly at the
onset of the fluidization. It is because the liquid circulation velocity is very low [16] if the fixed bed exist and the absorbed oxygen in the riser is very slowly distributed in the whole reactor. Approximately the almost whole absorbed oxygen accumulates in the riser i.e. similarly as in the bubble column. Instead of this if fluidization is started the liquid circulation immediately increases and oxygen is distributed in the liquid volume in the whole reactor volume. If the oxygen accumulation coefficient in the fixed bed reactor and in the fluidized bed will be compared taking into account the volume of the riser and the volume of the reactor it will be obtained

\[
\frac{k_{Ld}^{ac} u_{GR} \rightarrow u_{GRMF}^-}{k_{Ld}^{ac} u_{GR} \rightarrow u_{GRMF}^+} \approx \frac{0.0464 \text{ s}^{-1}}{0.0171 \text{ s}^{-1}} = \frac{A_R + A_D}{A_R} = 2.71
\]

The oxygen coefficient ratio value just before the fluidization \( u_{GRMF}^- \) and for velocity slightly higher than the minimum fluidization velocity \( u_{GRMF}^+ \) indicated by the equation (10) is almost equal to the ratio of the reactor volume to the riser volume.

5. Concluding remarks

The degradation course could be described by an equation similar to the first order reaction equation. The highest dimethylketone degradation rate in the presence of the Acinetobacter calcoaceticus bacteria immobilized on the carrier solid particles creating the inverse fluidized bed in the downcomer is observed for pH value about seven. It is worth to noticed that the most intensive growth of the bacteria A. calcoaceticus for named pH value is very convenient from the practical point of view taking into account a possible application for the waste water treatment.

The degradation rate constant increases if the gas flow rate increases in the reactor with the immobilized bacteria in the fixed bed. The degradation time decreases with an increase of the gas flow rate if the inverse fluidized bed exists. The approximately constant lowest value of the degradation time is observed if the gas flow rate is slightly higher than the minimum fluidization velocity value up to the about double minimum fluidization velocity value. The minimum fluidization velocity diminishes during the degradation time.

The degradation rate constant increases if the initial bed height of the bacteria carrier increases. It is obvious because the larger number of immobilized bacteria cover the surface of the fluidizing particles in the higher bed and it is too the larger organic substance - bacteria contact time.

The oxygen accumulation rate coefficient values in the inverse fluidized bed reactor in the reactor with bacteria are lower than that without bacteria. The oxygen accumulation rate coefficient apparently decreases distinctly at the onset of the fluidization. It caused by the distinct increase of the liquid circulation velocity in this point.
Symbols

- initial concentration of the dimethylketone, % vol
- height of the fluidized bed, m
- initial height of the packed bed, m
- correlation coefficient, -
- degradation constant , s⁻¹
- oxygen transfer coefficient, s⁻¹
- oxygen accumulation rate coefficient, s⁻¹
- dimethylketone concentration, kg.dm⁻³
- circulation time around the reactor loop, s
- liquid residence time in the fluidized bed during one circulation around the reactor loop, s
- total contact time bacteria – dimethylketone solution, s
- liquid circulation velocity, m.s⁻¹
- riser gas velocity at the onset of the fluidization, m.s⁻¹
- riser gas velocity, m.s⁻¹
- volumetric air flow rate, m³.s⁻¹

Subscripts and superscripts

- downcomer
- fluidized bed
- gas
- liquid
- riser
- solid

References

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