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ZALETY STOSOWANIA BIOREAKTORÓW FLUIDYZACYJNYCH W PROCESIE NITRYFIKACJI MIKROBIOLOGICZNEJ

ADVANTAGES OF THE APPLICATION OF FLUIDIZED BED BIOREACTORS TO A MICROBIOLOGICAL NITRIFICATION PROCESS

A b s t r a c t

The paper presents application possibilities of two and three-phase fluidized bed bioreactors for carrying out a microbiological nitrification process. The advantages and disadvantages of individual bioreactors are described. A quantitative analysis of the fundamental operating variables related to the balance of oxygen and the preservation of the fluidized bed was performed.

Keywords: *nitrification*, *bioreactor*, *fluidization*

S t r e s z c z e n i e

W artykule omówiono możliwości wykorzystania dwu- i trójfazowych bioreaktorów fluidyzacyjnych do realizacji nitryfikacji mikrobiologicznej. Przedstawione zostały zalety i wady poszczególnych rozwiązań procesowych. Dokonano ilościowej oceny podstawowych wielkości związanych z bilansem tlenu i utrzymaniem złoża w stanie fluidalnym.

Słowa kluczowe: *nitryfikacja*, *bioreaktor*, *fluidyzacja*

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Nomenclature

c – concentration [kg/m^3]
d_p – particle diameter [m]
F_V – volumetric flow rate [m^3/h]
H – fluidized bed height [m]
K – interfacial equilibrium constant [–]
Re_g – gas Reynolds number [–]
Re_{mf} – minimum fluidization Reynolds number [–]
u – velocity [m/s]
w_{TA} – yield coefficient [kg T/kg A]
α – conversion factor of carbon substrate [–]
η – dynamic viscosity coefficient [kg/(m×s)]
ξ – recirculation ratio [–]
ρ – density [kg/m^3]
τ_0 – liquid residence time in apparatus [h]

Subscripts

A – refers to carbon substrate A
m – refers to mixing node
mf – refers to minimum conditions of fluidization
r – refers to recirculation stream
T – refers to oxygen

Superscripts

c – refers to liquid phase

1. Introduction

Due to urbanization and developments in agriculture, ammonia nitrogen has become one of the key components of wastewater. The increased concentration of ammonia nitrogen has a negative influence on the quality of surface water. Ammonia in its un-ionized form is highly toxic to fish even at low concentrations of approximately 0.2 mg/l [1]. Futhermore, microbiological processes related to the oxygenation of ammonium ions can lead to a significant decrease of oxygen dissolved in water – this causes disturbances in the water biotic community [2].

Ammonia nitrogen which occurs in waters is formed as a result of the ammonification of substances contained both in municipal wastewater and agricultural leachate. An increase of nitrogen concentration in waters is also caused by the widespread use of fertilizers. Components of fertilizers dissolved in rain waters may then permeate into groundwater.

A separate problem connected with the content of ammonia nitrogen in water occurs in aquaculture. Owners of these cultures, which are dynamically evolving in different parts of the world, aim at reducing the usage of fresh water [3]. Therefore, it is necessary to use recirculating systems. Effluent water from aquaculture contains ammonia nitrogen

from metabolism and food waste. As mentioned above, ammonia nitrogen at relatively low concentrations is toxic to fish. Thus, it is crucial to remove ammonia nitrogen from the recirculated stream.

2. The nitrification process

The requirements for the quality of water destined for human consumption both in Poland [4] and in other countries [5, 6], impose constraints on concentrations of ammonia nitrogen, nitrite nitrogen and nitrate nitrogen. Similarly, an array of constraints on nitrogen compounds in effluents from wastewater treatment plants has been introduced. In these cases, excessive concentrations of nitrogen compounds could be dangerous for people or may be in violation of the law. While exceeding lethal nitrogen concentrations in aquaculture could lead to the dying out of a culture, this is not the limit of the problem as it also leads to financial losses.

Dangers resulting from increases in the ammonia or nitrite nitrogen concentration in water results in the removal of nitrogen compounds from water being a significant practical problem. The use of a microbiological nitrification process or sequence of nitrification-denitrification processes is a typical solution to this problem [2].

The nitrification process basically consists of two phases:

– oxidation of ammonium to nitrite

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H_2O + H^+ \tag{1}$$

– oxidation of nitrite to nitrate

$$NO_2^- + 0.5O_2H_2 \rightarrow NO_3^- \tag{2}$$

The phases of the nitrification process listed above take place with the participation of different genera of chemoautotrophic bacteria [7]. Chemoautotrophic bacteria are characterized by an ability to use inorganic compounds as a source of electrons.

The first phase of nitrification, during which ammonium is oxidized to nitrite, involves ammonia oxidizing bacteria (nitrite bacteria) of the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*. Oxidation of nitrite to nitrate takes place with the participation of bacteria of the genera *Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospina.* Both phases of the nitrification process take place in aerobic conditions and the molecular oxygen is an acceptor of electrons.

An analysis of the growth kinetics of nitrification bacteria demonstrates that they are characterized by a relatively small specific growth rate in comparison to heterotrophic bacteria. The biomass doubling of nitrification bacteria requires many hours. The small specific growth rate is of a big significance, because it determines requirements for design of bioreactors for carrying out the nitrification process. It is necessary for designed or applied apparatus to ensure a long enough mean residence time for micro-organisms.

Regarding the aerobic nature of the nitrification process, the content of oxygen in the process environment is of key importance. The theoretical demand for oxygen in the process amounts to 4.57 g O_2/g $N\text{-}NH_4^+$ [2]. A decreased content of oxygen can

lead not only to the limiting of the nitrification process, but also to the dying out of micro--organisms or stopping the process after the first phase which causes an accumulation of nitrite nitrogen.

The pH of the environment also has a vast influence on the process rate. Literature has it that the most beneficial value of pH is 7.8. The activity of nitrifying bacteria is highest in these conditions, but it decreases rapidly when pH falls below 7.0 [1].

Equation (1), which describes a simplified mechanism of the first stage of the nitrification process, shows that in this stage, hydronium ions are released into the process environment. Therefore, the progress of nitrification leads to the acidification of the environment. Maintaining a proper pH value of the reaction mixture is another important practical problem related to the realization of the nitrification process [8].

The activity of nitrifying bacteria is also influenced by other factors such as temperature or the presence of other chemical compounds which inhibit the nitrification process, e.g. chlorate (V) or sodium azide. The most effective growth of micro-organisms takes place at a temperature of ca. 25°C, but below 5°C and above 42°C, the growth rate of bacteria significantly decreases.

In summary, it is possible to list several problems connected with improved efficiency of carrying out the nitrification process through ensuring of the following:

- an appropriately long residence time of micro-organisms in the apparatus,
- the required quantity of oxygen necessary for carrying out the process,
- values of pH and temperature optimal for the process,
- the elimination of substances which have an inhibitory influence on the process.

In order to fulfill the process requirements, a key problem seems to be the selection of proper apparatus. The selection of an appropriate construction which will ensure the possibility of effectively carrying out the process, whilst at the same time avoiding costly maintenance and running, is a typical and, simultaneously, a nontrivial problem for the process engineer.

3. Nitrification in stirred tank reactors

In large wastewater treatment plants, a sequence of microbiological processes nitrification and denitrification is realized.

Historically, one of the first solutions constructed for the removal of nitrogen compounds was a system of two stirred tank bioreactors. One of these tanks is fed with oxygen in order to ensure proper oxygenation, while the other tank is not oxidized in order to ensure anaerobic conditions. In the technology of sewage treatment, two technological schemes are proposed based on stirred tank bioreactors. These schemes are presented in Fig. 1 [7].

The diagram presented in Fig. 1a provides a more intuitive explanation due to the order of the microbiological processes in which the sewage is treated. In this solution, nitrification precedes denitrification, while in Fig. 1b the order of processes is reversed. In both solutions, a partial recirculation of biomass is applied.

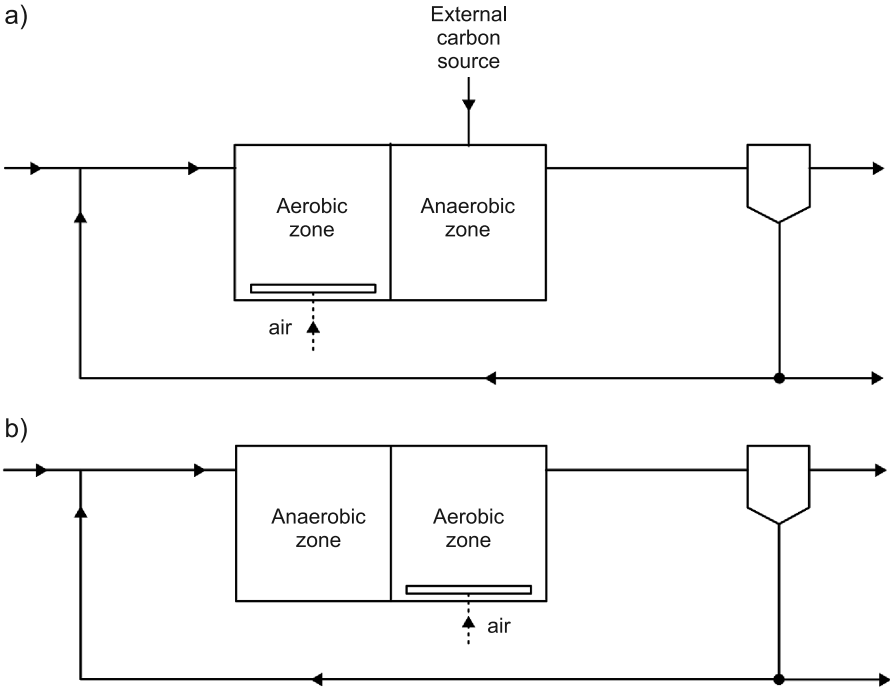

Fig. 1. Schematic diagrams of the nitrification-denitrification process in stirred tank bioreactors with recirculation

An aerobic zone in these systems, from the point of view of bioreaction engineering, is a bubble stirred tank bioreactor and an anaerobic zone is a perfect mixed tank bioreactor. In these types of bioreactors, micro-organisms can occur as both a suspended and an attached biomass. The mean residence time of suspended growth micro-organisms is equal to the residence time of sewage. An extension of the mean residence time of the biomass can be obtained by decreasing the liquid flow rate or by increasing the volume of the bioreactor. However, a decrease of flow rate is connected with a reduction in system productivity. The extension of the mean residence time together with maintenance of system productivity can only be obtained in practice by increasing the volume of the bioreactor. In reality, bioreactors of this type are substantially huge pieces of apparatus. In actuality, the indisputable advantages of this apparatus include its simple construction and low maintenance costs. From a practical point of view, a disadvantage of bubble stirred tank reactors is the lack of a possibility for an independent change of mean residence time of the biomass and liquid phase.

In aquaculture, where recirculation of large amounts of water is applied, a slightly different construction of the bioreactor can be used. This bioreactor enables the immobilization of micro-organisms on the large surface of the solid carrier.

Fixed packing is placed in a reactor. This packing is covered with a biofilm when the apparatus is in operation. There is a considerable interfacial surface between the liquid phase

and the biofilm phase, this results in an increase of mass transfer efficiency between these phases. The presence of a suspended growth biomass and that immobilized on the carrier leads to an increase in the general velocity of the nitrification process. A schematic diagram of such a solution is presented in Fig. 2.

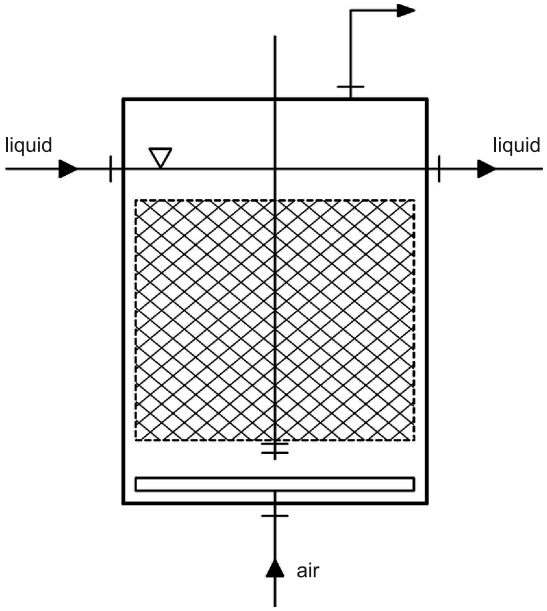

Fig. 2. Schematic diagram of the packed bed bioreactor

The immobilization of micro-organisms on the packing gives the possibility to separate the mean residence time of the liquid and the biomass. Therefore, the elimination of one of the key disadvantages of the bubble stirred tank bioreactor is possible.

Microbiological processes are usually relatively slow, this is why long mean residence times of reaction environment in the apparatus are necessary to carry them out. Appropriate values of the required residence time for the liquid phase can be obtained by reducing the flow rate, which, as mentioned above, results in a decrease in productivity. Additionally, hydrodynamic conditions inside the apparatus are also changed during this process. The shear stress acting on immobilized micro-organisms is lower in comparison to the case with higher flow rate. Low shear stress in the packed bed bioreactors can lead to the uncontrolled growth of the biomass and clogging of the bed.

The application of a packed bed provides the possibility of making the mean residence times of the liquid phase and the biomass independent, furthermore, it leads to an enlargement of the interface between the phases, but it can result in clogging of the bed. Additionally, the proposed solution with solid packing offers limited possibilities of control over the biofilm thickness in the apparatus. These features are considered to be the disadvantages of fixed bed bioreactors.

4. Fluidized bed bioreactors

The effect of bed clogging can be eliminated using fluidized bed bioreactors. In a fluidized bed, the shear stress is significantly higher than in a fixed bed reactor. Additionally, friction between the particles of the bed occurs. On one hand, these phenomena prevent the bed from clogging, but on other hand, they can cause the excessive shearing of biomass which leads to losses of micro-organisms and the slowing down of the microbiological process [9].

The application of biomass immobilization on small carrier particles in a fluidized bed also has important advantages, among which, the following should be pointed out [10]:

– obtaining a high total biomass concentration, which allows for the increasing of the overall process rate,
– the separation of the residence time of the liquid and biomass phases
– a large interphase contact surface between the liquid phase and the biofilm, which ensures a more efficient mass transfer,
– the possibility of constant bed exchange without stopping the process,
– a smaller size of apparatus in comparison to other solutions at the same productivity [11].

Mixing of the fluidized bed leads also to such a placement of bioparticles that lighter particles, on which the biofilm is thicker, appear in the upper part of the bed. This stratification gives the possibility of removing lighter bioparticles and controlling the thickness of the biofilm.

The application of fluidized bed bioreactors is also justified economically. The fluidized bed apparatus is characterized by the low unit costs of the mass transfer surface and the comparison of maintenance costs of different types of bioreactor shows that this type of apparatus is one of the cheapest [12].

Table 1

Examples of the application of fluidized bed bioreactors in aerobic degradation processes

No.	Substance	Micro-organisms	Carrier	References
1	phenol	Pseudomonas putida	activated carbon	Fan Liang-Shih et al. [14], Tang et al. [10]
2	dichloromethane	Pseudomonas, Methylobacterium, Hypomicrobium	charcoal	Gaelli [15]
3	phenoles, napthalenesulphonate	mixed culture	sand	Tijhuis et al. [16]
4	glucose	mixed culture	sand	Ryhiner et al. [17]
5	coal tar	mixed culture	sand	Hueppe et al. [18]
6	napthalenesulphonate	Pseudomonas	sand	Wagner and Hempel [19]
7	trichloroethene	mixed culture	activated carbon	Fennell et al. [20]
8	ammonia nitrogen	mixed culture	sand	Van Bentum et al. [21] Heijnen et al. [22]

The listed advantages of fluidized bed bioreactors make them more efficient than other solutions used in wastewater treatment technology [10, 13] and this has contributed to their considerable popularization. Examples of applications of fluidized bed bioreactors in aerobic degradation processes are presented in Table 1.

5. Nitrification in two and three-phase fluidized bed bioreactors

Fluidized bed bioreactors are often used to remove ammonia nitrogen. Apparatus of this type can be used in a relatively wide range of influent flow rates – this corresponds to the existence of a fluidized bed [11]. Due to the separation of the mean residence times of liquid and biomass, fluidized bed reactors can work at influent flow rates F_{Vf}, for which in bubble stirred tank bioreactors, the washout of biomass would occur.

By means of fluidized bed bioreactors, it is possible to remove as much as 90% of the ammonia nitrogen contained in the liquid phase in one reactor pass [12].

Nitrification as an aerobic process requires ensuring a relevant oxygen concentration in the bioreaction environment. A lack of sufficient oxygen in the environment can slow the process down and may also lead to the unfavorable phenomenon of the anoxia of micro--organisms.

In order to ensure a proper oxygenation, two solutions are applied in fluidized bed apparatus:

– two-phase liquid-solid fluidized bed bioreactor with an external aerator,
– three-phase gas-liquid-solid fluidized bed bioreactor.

A schematic diagram of a circulating fluidized bed bioreactor with external aeration is presented in Fig. 3. The role of an external mass exchanger is the oxygenation of a liquid stream directed to the bioreactor. External aerating apparatus ensures flexibility of the system with regard to the intensity of aeration, which depends on both substrate concentration and its flow rate.

One of the key process parameters crucial to the sufficient oxygenation of the liquid phase in a two-phase fluidized bed bioreactor is a recirculation ratio defined as:

$$\xi = \frac{F_{Vr}^{c}}{F_{V}^{c}} \tag{3}$$

A method of determination of a minimal value of this parameter based on oxygen requirement is presented below.

A mass balance of fresh and recirculated streams in mixing node gives

$$c_{Am}^{c} = (1-\xi)\, c_{Af}^{c} + \xi c_{A}^{c} \tag{4}$$

A relation between the quantity of consumed oxygen and substrate A, which undergoes oxygenation, is given by the following equation:

$$c_{T0}^{c} - c_{T}^{c} = w_{TA}(c_{Am}^{c} - c_{A}^{c}) \tag{5}$$

in which coefficient w_{TA} describes a quantity of oxygen used per mass unit of substrate A.

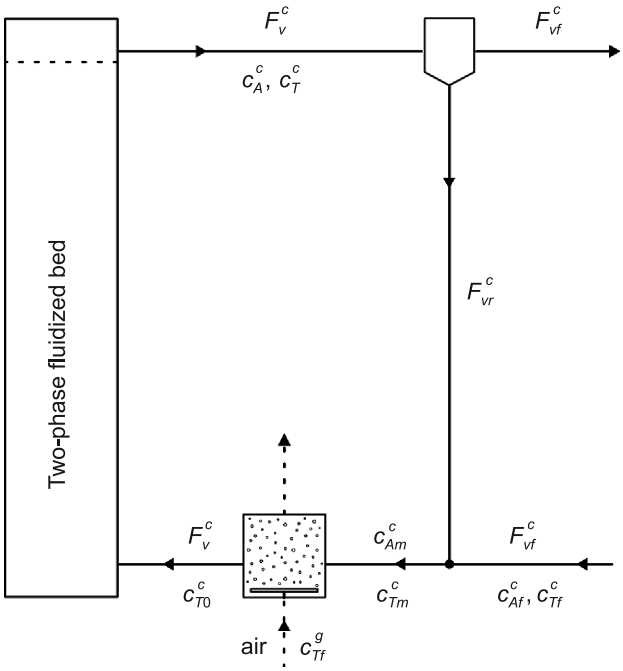

Fig. 3. Schematic diagram of a circulating fluidized bed bioreactor with an aerator in a recirculating loop

A minimal value of recirculation ratio $\xi_{\min}$ can be determined assuming that oxygen is totally consumed while passing through the reactor and that the aerator ensures complete oxygenation of the inlet stream, i.e. up to the point of saturation. These assumptions can be described with equation (6).

$$c_T^c = 0 \quad \text{and} \quad c_{T0}^c = c_T^* = \frac{c_{Tf}^g}{K} \tag{6}$$

Putting equation (6) into equations (4) and (5) we get:

$$\xi_{\min} = 1 - \frac{c_T^*}{w_{TA} c_{Af}^c \alpha} \tag{7}$$

where α is a conversion factor of substrate A defined as:

$$\alpha = \frac{c_{Af}^c - c_A^c}{c_{Af}^c} \tag{8}$$

Fig. 4a shows how value $\xi_{\min}$ forms for a few set values of the yield coefficient w_{TA}.

The application of fluidized bed bioreactors requires ensuring appropriate conditions for expanded fluidization. From the hydrodynamics of the fluidized bed, it follows that it can exist only in a strictly determined range of liquid velocity (between minimal

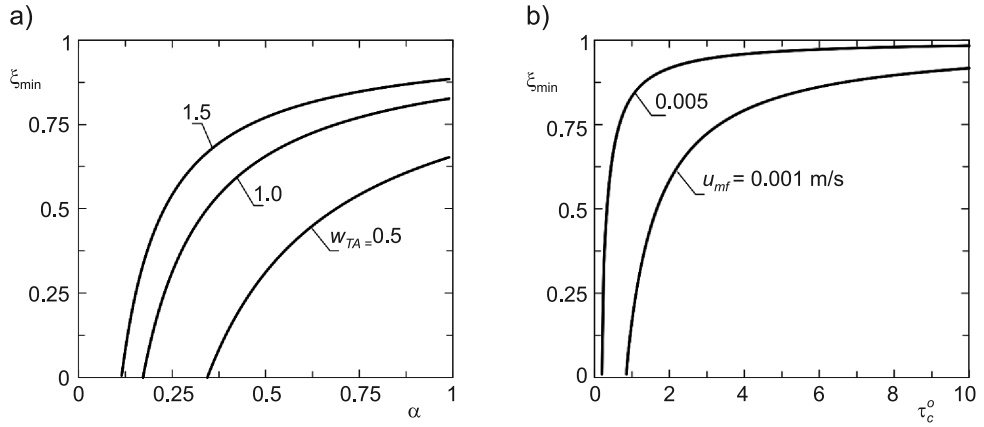

Fig. 4. Relationship of a minimal value of recycle ratio to: a) conversion factor for different values of the w_{TA} coefficient, b) the mean residence time of liquid for different values of u_{mf} ($c^c_{Af} = 0.05$ kg/m^3; $c^*_T = 0.0086$ kg/m^3)

fluidization velocity and the terminal velocity of the particles). Maintaining a proper liquid velocity at small inlet stream flow rates may demand high recirculation ratios. Therefore, the minimal recirculation ratio may also be related to the hydrodynamics of the fluidized bed. The relationship between the value of the recirculation ratio, the minimal fluidization velocity u_{mf}, the height of the bed in apparatus H and the mean residence time of liquid τ_0^c is described by equation (9):

$$\xi_{\min} = 1 - \frac{H}{u_{mf}\tau_0^c} \tag{9}$$

A relationship between $\xi_{\min}$, which was calculated as shown, and the mean residence time of liquid in the system τ_0^c for two chosen values of u_{mf} is presented in Fig. 4b.

While designing the bioreactor shown in Fig. 3, a bigger value out of these $\xi_{\min}$ calculated according to formulas (7) and (9) is mandatory.

As an alternative solution to two-phase bioreactors, three-phase fluidization is applied. Apart from liquid, gas is also supplied to the bioreactor. A schematic diagram of such a system is presented in Fig. 5.

The presence of gas and its flow velocity have a large influence on the minimal fluidized bed velocity of particles. In Fig. 6, results obtained by Macchi and co-authors [23] are presented. The points on the figure represent experimental data. The curves are plots of approximation correlations obtained as a part of this work. The results achieved by Macchi are described by the following equations:

a) for air-glycerol-glass system: $\mathrm{Re}_{mf} = 31.31\,\mathrm{Re}_g^{-0.3033}$, at a value of regression coefficient $R^2 = 0.999$,

b) for air-silicone oil-alumina system: $\mathrm{Re}_{mf} = 29.82\,\mathrm{Re}_g^{-0.2461}$, at a value of regression coefficient $R^2 = 0.994$.

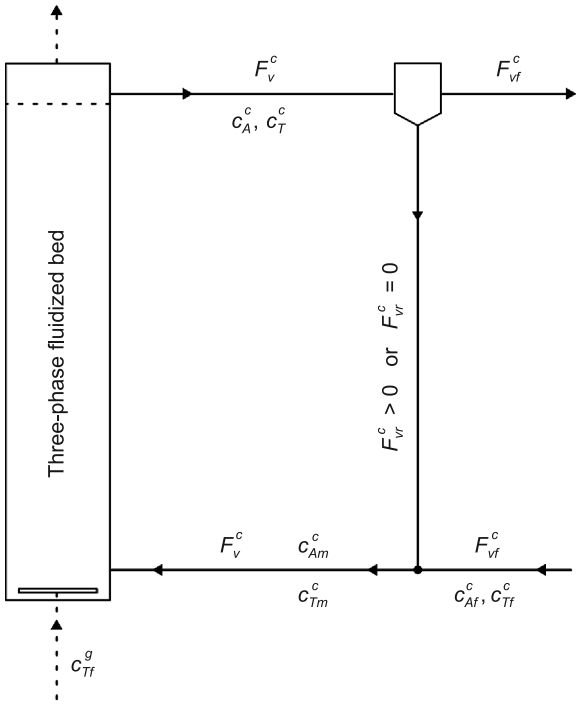

Fig. 5. Schematic diagram of a three-phase gas-liquid-solid fluidized bed bioreactor with partial thickening and recirculation of the biomass

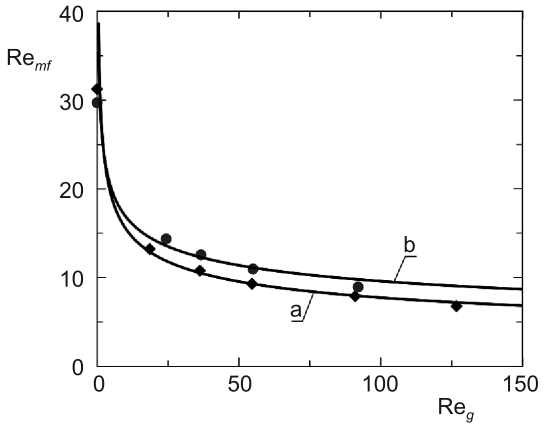

Fig. 6. Relationship of minimum fluidization Reynolds number to gas Reynolds number: a) for air-glycerol-glass system, b) for air-silicone oil-alumina system

Definitions of Reynolds numbers are presented by equations (10).

$$\mathrm{Re}_{mf} = \frac{u_{mf} d_p \rho_c}{\eta_c} \qquad \mathrm{Re}_g = \frac{u_g d_p \rho_c}{\eta_c} \tag{10}$$

According to equation (9), a lower value of minimum fluidization velocity u_{mf} corresponds to a lower minimal recirculation ratio. Thus, from the hydrodynamics point of view, at appropriately high values of gas velocities, liquid recirculation will not be necessary to maintain the fluidized bed.

Air bubbles, which appear in three-phase bioreactors, exert shear stress which leads to the intensification of biomass detachment from low-density particles [24]. Furthermore, air bubble coalescence inside a bioreactor may occur, which results in bubbles raising too quickly and worsening of conditions of mass transfer between liquid and gas.

According to Tang and Fan [10, 14], airlift three-phase bioreactors are also treated as fluidized bed apparatus. A schematic diagram of a piece of apparatus of this type is presented in Fig. 7. Circulation in airlift fluidized bed apparatus is as a result of the density difference of fluids in a riser and downcomer. Therefore, the application of additional pumps in order to force circulation is not necessary – this is a significant advantage of this solution.

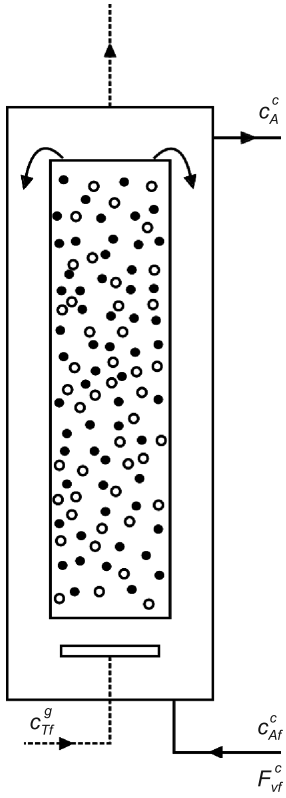

Fig. 7. Schematic diagram of three-phase airlift apparatus

The presence of gas bubbles and liquid circulation ensures effective mixing of the reaction environment. Airlift bioreactors are also used for carrying out the microbiological nitrification process [25, 26]. The conversion factor of ammonia nitrogen during nitrification in a bioreactor of this type may amount to as much as 0.99 [27]. However, it is worth mentioning that because of the operation principle of airlift bioreactors based on density difference of fluids in different zones, this apparatus has to be relatively high.

The design of fluidized bed reactors is relatively complex [12]. One of the key problems is the selection of a proper diameter, material and mass fraction of particles of a fluidized bed [28].

In the fluidized apparatus described above, gas and liquid flow in the same direction, i.e. the bioreactor is fed with these materials from the bottom. Introducing liquid feeding from the top of the apparatus leads to the formation of an inversed fluidized bed. The application of an inversed fluidized bed gives the possibility of limiting shear stress in the bed, a greater control of the biofilm thickness and prevents particles from being carried up [29]. With regard to the hydrodynamics of the inversed fluidized bed, it is necessary to use particles of slightly lower density than that of liquid phase. Polymers are usually used as carriers of micro-organisms in this type of bed.

6. Fluidized beds with chemically active particles

Equation (1) shows that in the first stage of the nitrification process, protons are released to the reaction environment. The hydronium ions which are formed cause a decrease in the pH of the environment. As it was mentioned above (point 2) a level of pH below neutral leads to a rapid reducing of the growth rate of the nitrifying bacteria, which results in a lower overall efficiency of the process. Maintaining the proper pH in a reaction environment is possible due to the dosage of appropriate chemical compounds, e.g. carbonates or hydroxides. Using a specific compound, which would make the environment neutral, as carrier in the fluidized bed could be a cheaper and more rational solution. In literature [8], chalk is suggested as a carrier. From the chemical point of view, chalk is mainly calcium carbonate. Chalk may be successfully used as a carrier biofilm and it simultaneously allows for the neutralization of hydronium ions formed during the nitrification process. A drawback to this solution is the necessity of filling in chalk losses during operation of the apparatus. Additionally, from the design point of view, it is necessary to take into consideration the kinetics of chalk decomposition. However, it does not constitute a disadvantage of the process because the application of chalk as a biofilm carrier is in this case, economically justified.

7. Conclusions

The processes of ammonia nitrogen removal from municipal and industrial wastewater have a serious influence on the improvement of parameters of effluents from wastewater treatment plants. Furthermore, they are also useful in the treatment of water for human consumption. In some branches of agriculture, such as aquaculture, they are necessary for the rational management of surface waters.

The biological removal of nitrogen compounds may be carried out in different types of bioreactors. In practice, both large continuous stirred tank bioreactors and the more efficient fluidized bed apparatuses are used.

From a process engineering point of view, it is more advantageous to apply fluidized bed bioreactors due to the higher overall biomass concentration, lack of bed clogging, separation of residence times of liquid and biomass, intensification of mass transfer between liquid and biofilm and the possibility of controlling the thickness and age of the biofilm.

The literature shows the application of both two-phase (liquid-solid) and three-phase (gas-liquid-solid) bioreactors applied for the nitrification process. These solutions and the significance of the recirculation ratio in fluidized bed bioreactors are presented in this work, as well as a method of determination of minimal recirculation ratio is proposed.

It is worth noting that fluidized bed bioreactors are smaller in relation to other solutions with the same productivity, which is an additional advantage. The application of fluidized bed apparatuses, apart from process advantages, is also economically justified because they have relatively low maintenance costs.

The fluidized bed allows for the filling in of particles during the operation of the bioreactor which makes the controlling of the biofilm age easier and provides the possibility of using chemically active particles. In the case of the microbiological nitrification process, it is necessary to ensure the proper pH of the environment, which may be practically obtained by using chalk particles as the biofilm carrier. Then the particles neutralize the formed hydronium ions and allow to ensure the constant value of pH of the environment.

In summary, carrying out the microbiological nitrification process in fluidized bed bioreactors is justified both from practical point of view and economically, but the selection of specific apparatus demands a deeper quantitative analysis related to the studied design case.

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