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Biodegradation of hexavalent chromium (Cr^{+6}) in wastewater using Pseudomonas sp. and Bacillus sp. bacterial strains

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Abstract
The recovery of toxic metal compounds is a deep concern in all industries. Hexavalent chromium is particularly worrying because of its toxic influence on human health. In this paper, biodegradation of hexavalent chromium (Cr^{+6}) present in wastewater has been studied using two different bacterial strains; Pseudomonas sp. and Bacillus sp. A chemostat (with and without recycle of cells) with 10 L liquid culture volume was used to study the substrate and the biomass cell concentrations with time. Also, the degree of substrate conversion was studied by the varying the dilution rate as an independent parameter. The dilution rate (ratio of feed flow rate to the culture volume) was varied by varying the feed volumetric rate from 110-170 mL/h for inlet hexavalent chromium concentrations of 70 mg/dm^3. The results show that a chemostat with recycle gives a better performance in terms of substrate conversion than a chemostat without a recycle. Moreover, the degree of substrate conversion decreases as the dilution rate is increased. Also, Bacillus sp. was found to give higher conversions compared to pseudomonas sp.

Keywords: Chemostat; Hexavalent chromium; Biodegradation; Dilution rate; Bacillus; Pseudomonas.

1. Introduction

Water contamination by heavy metals is one of the most noteworthy problems in the current century. Heavy metals such as lead, mercury, chromium, copper and nickel are highly toxic even at very low concentrations. The rapid growth in water pollution by industries requires effective measures to reduce environmental degradation.

Among the various heavy metals, hexavalent chromium (Cr^{+6}) has been found in harmful concentrations in surface waters due to contamination introduced from industrial pollution. In fact, hexavalent chromium is highly water soluble with a solubility of 1680 g/L water [1]. This high solubility of hexavalent chromium in water makes it a 'priority pollutant' as indicated by the U.S. Environmental Protection Agency [2]. The trivalent chromium (Cr^{+3}) does not represent a major problem since its solubility in water is low and can be easily separated from water. Also, trivalent chromium is 100 times less toxic than hexavalent chromium [2].

There are numerous sources of introduction of hexagonal chromium into the environment. Hexavalent chromium in industrial wastewater mainly originates from important industrial processes such as electroplating, chromate manufacturing, dyes and pigment manufacturing, wood preservation, leather tanning industry, manufacture of alloys and as corrosion inhibitor in conventional and nuclear power

plants [2]. Moreover, Petroleum refining processes have also resulted in introduction of Cr^{+6} into soil, air and water [3].

Among the various methods to remove hexavalent chromium from wastewater, biodegradation of hexavalent chromium by microorganisms in a biological continuous stirred tank reactor (CSTR), known as a chemostat, can also be used. Many aerobic and anaerobic microorganisms are capable of reducing Cr^{+6} to Cr^{+3}, which consume Cr^{+6} as a nutrient to grow. Two such bacterial strains will be studied in this paper namely, Pseudomonas sp. and Bacillus sp. Finally, due to low solubility of Cr^{+3}, it can be removed easily from water.

In this paper, the biodegradation of Cr^{+6} will be studied in a chemostat. The culture of microorganisms is placed inside the chemostat and fresh feed containing the substrate is continuously added to the culture medium. The volume of the culture in the chemostat is kept constant by continuously removing the culture liquid. Since aerobic microorganisms require oxygen for their growth, a chemostat may contain an oxygen sparger to supply oxygen to the microorganism necessary for their growth. As the bio reaction progresses, the substrate is consumed by the cells. The cells grow in size and multiply and may also produce useful products (such as antibiotics) [4]. Figure 1 shows a typical chemostat. This paper covers the study and application of biodegradation of Cr^{+6} using bacterial cells in a chemostat. The degree of conversion of the substrate (Cr^{+6}) will be studied using two different chemostat configurations (with and without cell recycle) and the effect of dilution rate on conversion will be highlighted.

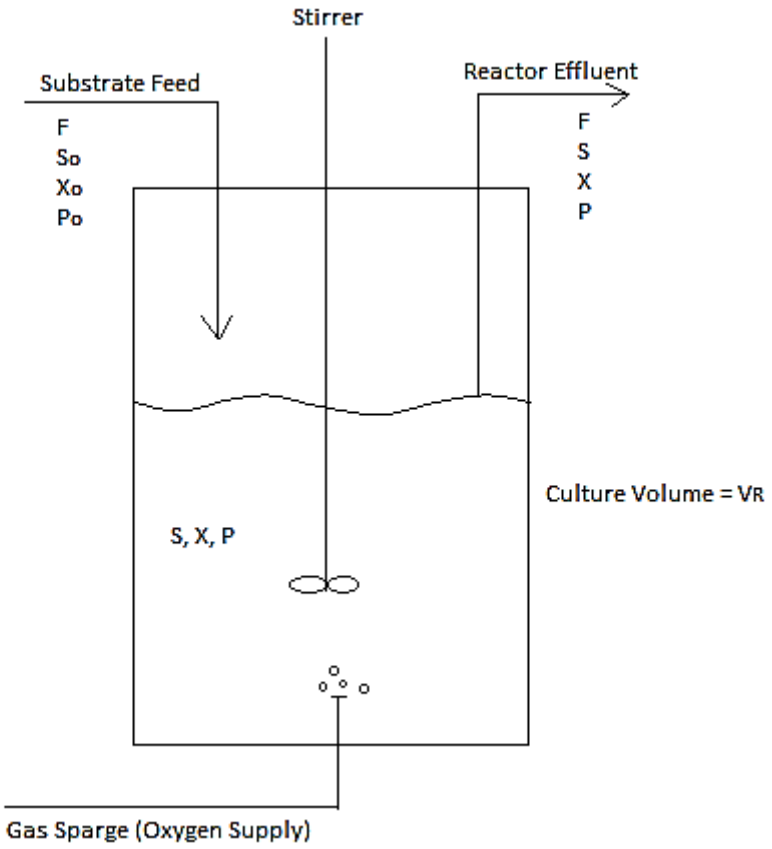

Figure 1. A simple chemostat

2. Theory

2.1 Kinetic parameters

The bioconversion reaction of Cr^{+6} to Cr^{+3} is inhibited by the substrate when the former is used at a higher concentration level. Since in this study the range of initial concentration of the substrate used will be fairly high (70 mg/dm^3), the reaction will be assumed to be inhibited by the initial Cr^{+6} concentration. The classical substrate inhibited Haldane equation for microbial cell growth rate is given by [5]:

$$\mu_g = \frac{\mu_{\max} S}{K_S + S + \frac{S^2}{K_i}} \quad (1)$$

where: S is the concentration of the substrate (Cr^{+6}) in the chemostat (mg/dm^3), μ_{max} is the maximum specific microbial cell growth rate (h^{-1}), K_S is the Monod constant (mg/dm^3), and K_i is the inhibition constant.
In this study, the kinetic parameters for the two bacterial microorganisms are presented in Table 1 [5]:

Table 1. Kinetic parameters for equation 1

Type of Bacteria	μ_{max} (h^{-1})	K_S (mg/dm^3)	K_i (mg/dm^3)	$Y_{X/S}$
Pseudomonas sp.	0.046	16.32	200.37	0.665
Bacillus sp.	0.056	9.43	305.29	0.776

In Table 1, $Y_{X/S}$ represents the growth yield coefficient, that is, the amount of cells (X) produced per unit of substrate (S) consumed.

2.2 Chemostat model without recycle
Let S and X denote the concentration of the substrate (Cr^{+6}) and biomass at any time in the chemostat respectively, and V_R denote the liquid culture volume in the chemostat. A material balance on the cell concentration around the chemostat in Figure 1 yields:

$$FX_0 - FX + V_R \mu_g X - V_R k_d X = V_R \frac{dX}{dt} \quad (2)$$

where, μ_g is the growth rate given by Eq. (1), k_d is the death rate, which is assumed to be zero in this case (k_d=0), F is the feed flow rate, which is equal to the effluent flow rate, V_R is the constant bacterial culture volume, X_0 and X is the concentration of the cells in the feed and in the chemostat, respectively. Eq. (3) can be written as:

$$DX_0 - DX + \mu_g X - k_d X = \frac{dX}{dt} \quad (3)$$

where,

$$D = \frac{F}{V_R} = \text{Dilution Rate} \quad (4)$$

The dilution rate is simply the reciprocal of the residence time and is an important parameter for bioreactors. Assuming the death rate to be zero (only growing cells), and sterile feed (no cells in the feed solution, X_0=0) the final form of the cell mass balance is:

$$\frac{dX}{dt} = (\mu_g - D)X \quad (5)$$

Substituting Eq. (1) into Eq. (5), we get:

$$\frac{dX}{dt} = \left[\frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_i}} - D \right] X \quad , \quad X(0) = X_0 = 10 \frac{mg}{dm^3} \quad (6)$$

The dilution rate (D) is a constant that depends on the liquid volume in the chemostat and the feed solution flow rate. Usually, the dilution rate can only be increased up to a certain limit to avoid wash out of the cells. At wash out, the flow rate becomes so large that the cells are carried out of the chemostat and cell concentration in the chemostat becomes zero. If D is set to a very large value, the culture cells cannot reproduce quickly enough and are washed out. High dilution rates make the residence time very small and thus, the cells spend very small amount of time in the chemostat and do not grow properly. In this study, the initial concentration of the cells in the chemostat is assumed to be 10 mg/dm^3 [5].
The substrate (Cr^{+6}) consumed by the microorganisms is utilized for cellular growth and to make extracellular products (if any). In this case, product formation by the cells is neglected; therefore, the mass balance on the substrate is given by the following equation (no substrate generation):

$$FS_0 - FS - \frac{V_R \mu_g X}{Y_{X/S}} = V_R \frac{dS}{dt} \tag{7}$$

Eq. (7) can then be written as:

$$D(S_0 - S) - \frac{\mu_g X}{Y_{X/S}} = \frac{dS}{dt} \tag{8}$$

Finally, substituting Eq. (1) into Eq. (8) we get:

$$\frac{dS}{dt} = D(S_0 - S) - \left(\frac{X}{Y_{X/S}} \frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_i}} \right), S(0) = S_0 = 70 \frac{mg}{dm^3} \tag{9}$$

The initial condition assumes that initially the concentration of the substrate in the chemostat is 70 mg/dm^3. Equations 6 and 9 are the two coupled ODEs that represent mass balance for the substrate (Cr^{+6}) and the biomass cells.

2.3 Chemostat model with recycle

Since microbial conversions are autocatalytic (the cells consume the substrate and more cells are produced), the rate of conversion of the substrate (Cr^{+6}) can be increased by increasing the microbial cell concentration in the chemostat. This can be achieved by recycling the cells in the chemostat effluent back to the reactor. In fact, cell recycle also increases the stability of waste-water treatment processes by minimizing the effects of process perturbations [4]. Figure 2 shows the modified chemostat in which a cell recycle is used. A material balance on the biomass (bacterial cells) around the chemostat in the above Figure 2 gives the following equation:

$$FX_0 + \alpha FCX_1 - (1+\alpha)FX_1 + V_R \mu_g X_1 - V_R k_d X_1 = V_R \frac{dX_1}{dt} \tag{10}$$

where, α is the recycle ratio based on volumetric flow rates, C is the concentration factor, X_2 is the concentration of cells in effluent from the cell separator. Again, Eq. (10) can also be written as (for no cell death, k_d=0 and sterile feed X_0=0):

$$\alpha DCX_1 - (1+\alpha)DX_1 + \mu_g X_1 = \frac{dX_1}{dt} \tag{11}$$

Substituting the expression for μ_g from Eq. (1) into Eq. (11), we get:

$$\frac{dX_1}{dt} = \left[\alpha DC - (1+\alpha)D + \frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_i}} \right] X_1,\ X_1(0) = 10 \frac{mg}{dm^3} \tag{12}$$

Similarly, a material balance on the substrate (Cr^{+6}) gives:

$$FS_0 + \alpha FS - (1+\alpha)FS - \frac{V_R \mu_g X_1}{Y_{X/S}} = V_R \frac{dS}{dt} \tag{13}$$

Eq. (14) can then be written as:

$$D(S_0 - S) - \frac{\mu_g X_1}{Y_{X/S}} = \frac{dS}{dt} \tag{14}$$

Finally, substituting Eq. (1) into Eq. (8) we get:

$$\frac{dS}{dt} = D(S_0 - S) - \left(\frac{X_1}{Y_{X/S}} \frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_i}} \right),\ S(0) = 70 \frac{mg}{dm^3} \tag{15}$$

Equations 12 and 15 are the two coupled ODEs that represent mass balance for the substrate (Cr^{+6}) and the biomass or bacterial cells.

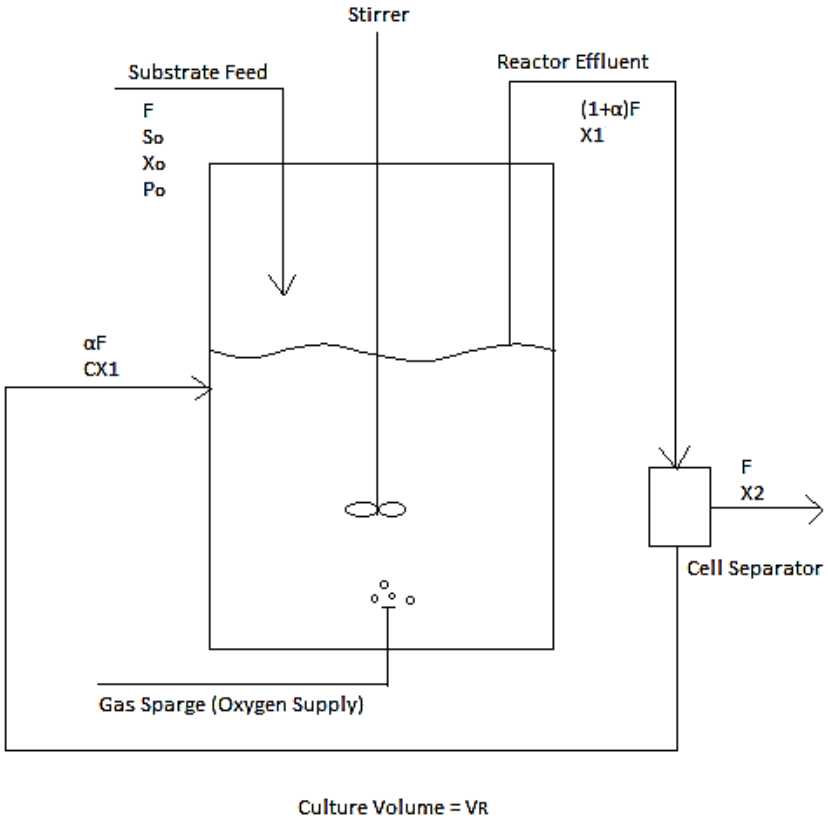

Figure 2. Chemostat with recycle

3. Results and discussion

The differential equations derived were solved using 4th order Runge-Kutta method in MATLAB. The main parameter under study was the dilution rate (D). The dilution rate was varied by changing the inlet flow rate (F) to the chemostat, while the reactor culture volume (V_R) was kept constant at 10 dm^3. The dilution rate was varied from 0.011 h^{-1} to 0.017 h^{-1}. The inlet substrate (Cr^{+6}) concentration S_0 was set at 70 mg/dm^3 [5]. Moreover, for a chemostat with a recycle, the recycle ratio (α) in Eq. (12) and Eq. (15) was fixed at 0.3 while the concentration factor (C) was fixed at 2. Eq. (6) and Eq. (9) were solved directly using MATLAB solver ode45 which is based on an explicit Runge-Kutta (4,6) formula, the Dormand-Price pair.

As expected, Figures 3-6 for a chemostat without recycle and Figures 7-10 for a chemostat with recycle show that the bacterial cells grow by consuming the substrate Cr^{+6}. Therefore, the substrate concentration decreases with time and the cell concentration in the reactor increases with time.

The type of bacterial cells was found to have an important effect on the degree of substrate conversion. It was found that at any dilution rate and given reactor configuration (with or without recycle), Bacillus sp. bacterial strains achieved a higher degree of substrate conversion than Pseudomonas sp.

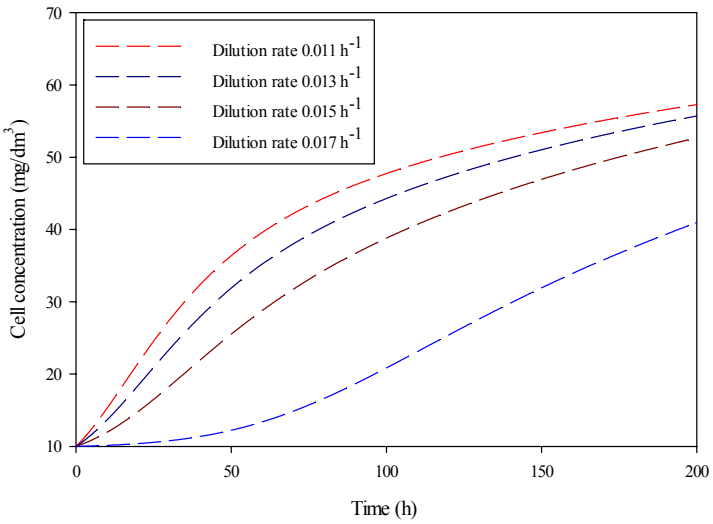

Figure 3. Cell concentration as function of time and dilution rate (Chemostat without recycle, Pseudomonas sp.)

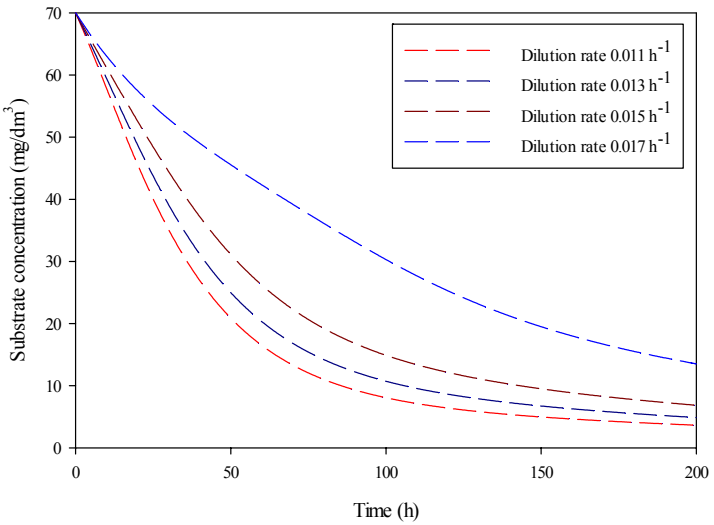

Figure 4. Substrate concentration as function of time and dilution rate (Chemostat without recycle, Pseudomonas sp.)

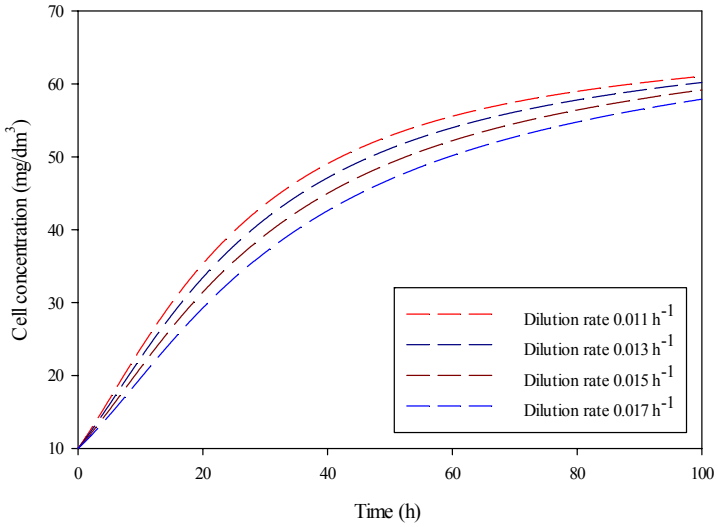

Figure 5. Cell concentration as function of time and dilution rate (Chemostat without recycle, Bacillus sp.)

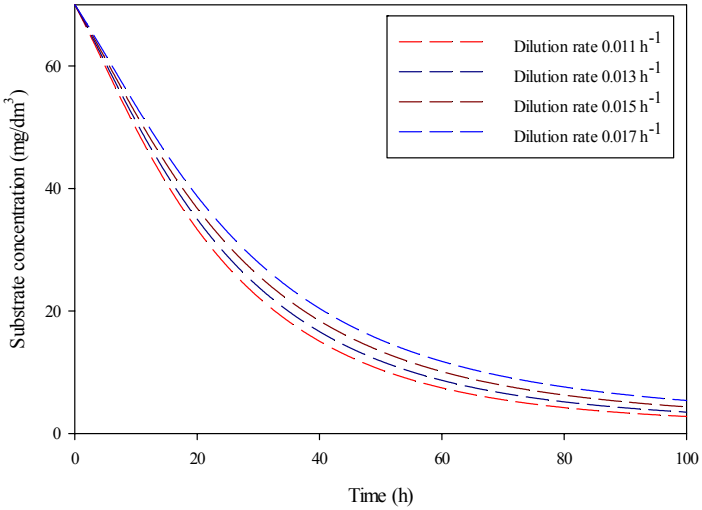

Figure 6. Substrate concentration as function of time and dilution rate (Chemostat without recycle, Bacillus sp.)

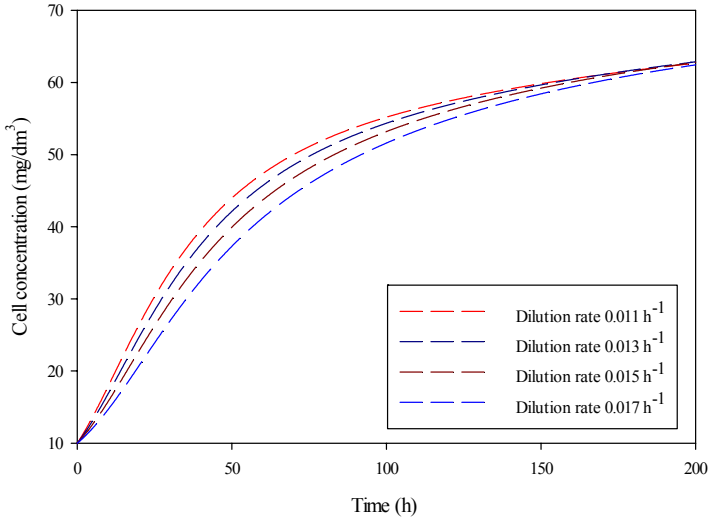

Figure 7. Cell concentration as function of time and dilution rate (Chemostat with recycle, Pseudomonas sp.)

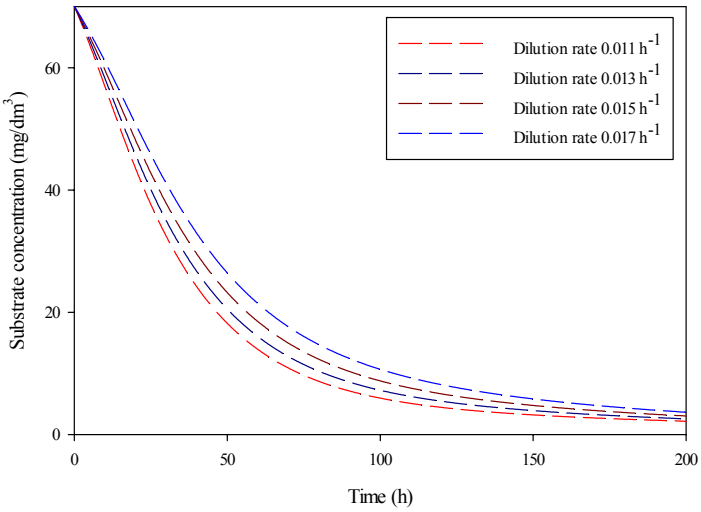

Figure 8. Substrate concentration as function of time and dilution rate (Chemostat with Recycle, Pseudomonas sp.)

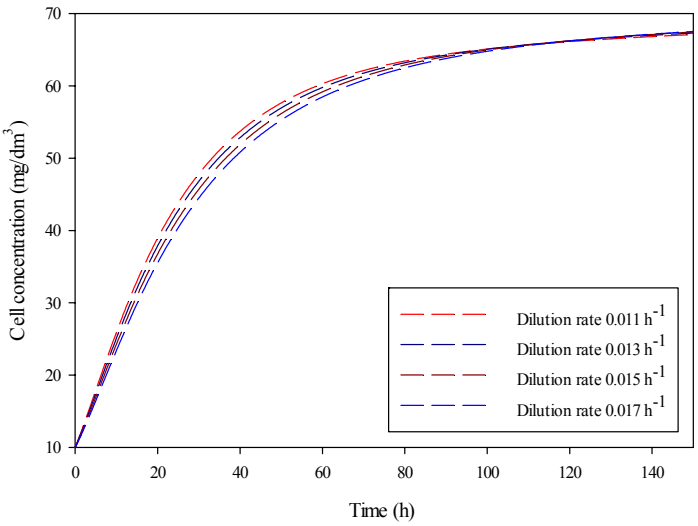

Figure 9. Cell concentration as function of time and dilution rate (Chemostat with Recycle, Bacillus sp.)

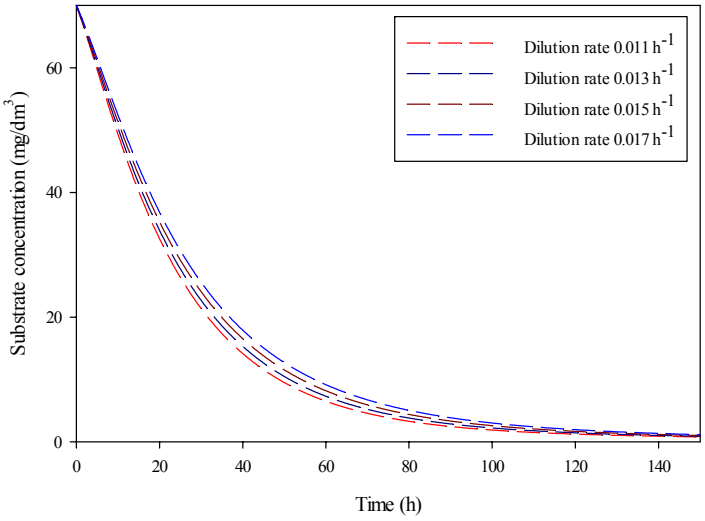

Figure 10. Substrate concentration as function of time and dilution rate (Chemostat with Recycle, Bacillus sp.)

Therefore, it is preferred to use Bacillus bacterial strains to remove Cr^{+6} from wastewater since a higher degree of substrate conversion can be achieved.

The results, as indicated by Figure 11, show that the conversion increases as cells are recycle back to the reactor. The reason behind this is that as more cells are added to the reactor by the recycle, more of the substrate will be consumed by greater number of bacterial cells. Therefore, we expect the cell concentration to be higher and the substrate concentration to be lower for a chemostat with recycle operating at steady state.

The dilution rate was also found to have a profound effect on the degree of substrate conversion. Figure 11 shows that as the dilution rate is increased the degree of substrate conversion decreases. This is because on increasing the dilution rate, the feed flow rate increases and the bacterial cells do not have enough time to grow inside the reactor. The higher flow rate will decrease the residence time of the cells in the reactor, thus, decreasing the substrate consumption.

In fact, if the dilution rate is set to a very large value, it is expected that the cells inside the reactor will be washed out and the conversion will drop to zero. Therefore, there is an optimum value of the dilution rate that must be determined.

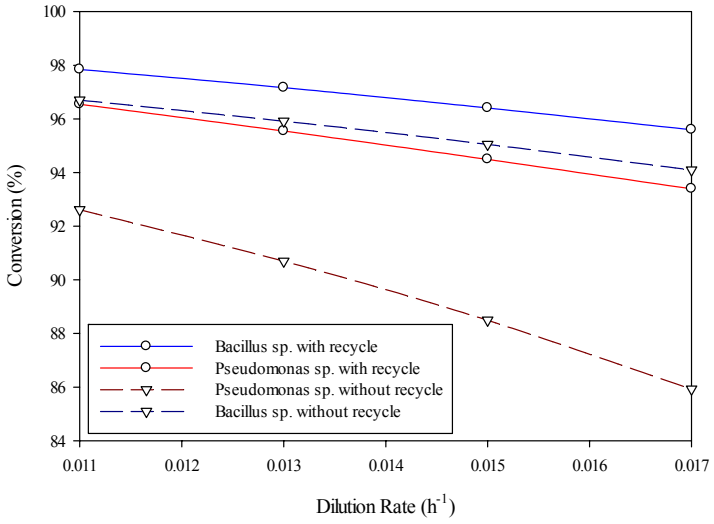

Figure 11. Conversion vs. dilution rate

4. Conclusion

From the results, it can be concluded that biodegradation of hexavalent chromium in wastewater can be done successfully using a chemostat. It is better to use a cell recycle in order to obtain higher substrate conversion levels. The dilution rate is was found to have a significant effect on the level of substrate conversion. Higher dilution rates resulted in lower substrate conversion. Among the two bacterial strains used, it was found that Bacillus sp. strain achieved a higher conversion than Pseudomonas sp. strain regardless of the chemostat configuration. In short, lower dilution rates, cell recycle, and use of Bacillus sp. bacterial strain were found to be the best combinations in order to achieve the highest substrate conversion level.

Acknowledgements

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