**ARAŞTIRMA MAKALESİ**

# MIXING-MODELING OF A COMBINED TWO-REACTORS SYSTEM FOR SCALE-DOWN APPROACHES IN BIOREACTORS

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**BİYOREAKTÖRLERDEKİ YETERSİZ KARIŞMANIN KOMBİNE İKİ REAKTÖR SİSTEMİ KULLANILARAK MODELLENMESİ**

**ÖZET**

Genel olarak, endüstriyel çaptaki reaktörlerde oluşan akış davranışları, piston akış ve tamamen karışmış akış olarak ikiye ayrılır. Biyoproses mühendisliği araştırmalarında karışma ile ilgili çalışmaları modellemek için kombine reaktör sistemleri, model sistemi olarak kullanılır. Bu çalışmada, endüstriyel çaptaki reaktörde meydana gelen iki farklı karışma davranışının simülasyonu için sürekli karıştırmalı tank reaktör (CSTR) ile piston akışlı boru reaktörün (PFTR) kombinasyonundan yararlanılmıştır. Burada endüstriyel çaptaki reaktörde meydana gelebilecek iyi karışan bölge ile yetersiz karışan bölge; kombine reaktör sistemi kullanılarak; sürekli karıştırmalı tank reaktör (CSTR) tamamen karışan bölgeyi, piston akışlı boru reaktör (PFTR) ise yetersiz karışan bölgeyi temsil etmektedir. Piston akışlı boru reaktördeki farklı kalma zamanlarının etkilerini incelemek için, bir pompa kullanılarak sirkülasyon hızı değiştirilmiştir. Kombine sistem için matematiksel model kurulmuş ve daha sonrada metilen mavisi kullanılarak deneyler gerçekleştirilmiştir. Kombine sistemdeki piston akışlı boru reaktör (PFTR) için geri karışmanın derecesi (Peklet sayısı) farklı pompa sirkülasyon hızları için tespit edilmiştir. Bu çalışmada yapılan teorik ve deneysel çalışmalar sonucunda, elde edilen datalar birbiri ile karşılaştırılmış ve yakın değerler elde edildiği gözlenmiştir.

**Anahtar kelimeler:** Yetersiz karışma, kombine biyoreaktörler, Peklet sayıları

**SUMMARY**

Generally, flow behaviour of the industrial reactors deviates from the two limiting conditions of plug-flow and completely mixed flow. To simulate such mixing conditions, a combined two reactors system is required in bioprocess engineering research as a model system for mixing studies. In this paper, the combined two reactors system consists of a continuous stirred tank reactor (CSTR) and a plug flow tubular reactor (PFTR) connected with a circulation flow of varied rate in order to realize different residence times in circulation. A mathematical model of this system is presented and compared with the tracer experiments by using a dye (methylene blue). The degree of backmixing (Peclet number) in the tubular section of the combined system is estimated for different recirculating pump rates. A good agreement between the theoretical and experimental results is obtained.

**Key words:** Imperfect mixing, combined bioreactors, Peclet numbers

## 1. INTRODUCTION

Scale-up is considered to be a major bottleneck in fermentation technology. This is predominantly due to the near impossibility of reproducing the ideal conditions obtained in the small fermenters used for research in the much larger production vessels. In the small-scale laboratory fermenters, mixing is near perfect. With mixing times within such a system being only a second or so, there will be little or no environmental heterogeneity. As the scale of the system increases, heterogeneity of the environment will become more apparent. In large-scale vessels, the mixing time can be over 100 seconds, resulting in mixing being far from ideal (Kristiansen [1]). This alters performance of the cells as the cells respond to changing environmental conditions through which they pass in the circulation loop. Therefore, mixing time can be a significant parameter that governs the productivity of large vessels.

Flow behaviour of a majority of the actual reactors (STRs or CSTRs) deviates from the two limiting conditions of plug-flow and completely mixed flow. The deviation from completely mixed flow may be caused by non-uniform velocity profile, velocity fluctuation due to molecular or turbulent diffusion, by short-circuiting, by-passing and channeling of fluid, by the presence of stagnant regions of fluid caused by the reactor shape and internals, or by the recycling of fluid within the reactor as a result of agitation. However, several flow models have been studied by the other workers [2-12] and all involve the subdivision of the bioreactor into a number of regions where the mixing characteristics are different.

In this present work, a poorly mixed large-scale reactor was simulated by using combined two reactors system (CSTR+PFTR). Hence, two mixing regions were created as good mixing (in the CSTR) and poor mixing (in the PFTR). Mathematical modelling of this system was derived, and the theoretical results were compared with the results of tracer experiments. To determine some degree of backmixing (Peclet numbers) in the PFTR section of the system, a tracer (methylene blue) was added to the combined two reactors system, and the concentration change was followed against time, then Peclet numbers were estimated by using parameter estimation program for different pump rates.

## 2. MATHEMATICAL MODELLING OF THE SYSTEM

For the mathematical modelling to describe a combined two reactors system (see Figure 1), the basic equations were developed by writing the dye (methylene blue) balance equations for the backmix and tubular sections, taking into account the inlet and outlet positions.

$$\frac{dC_B}{dt} = \frac{fr}{V_B} C_Z - \frac{(f + fr)}{V_B} C_B, \text{ for CSTR section} \quad (1)$$

$$\frac{dC_Z}{dt} = E_Z \frac{d^2 C_Z}{dz^2} - U \frac{dC_Z}{dz}, \text{ for PFTR section} \quad (2)$$

$E_Z$ refers to axial dispersion coefficient. In equation 2, the radial dispersion was neglected because the ratio of column diameter to length was very small (Levenspiel [13], Wen [14]). The numerical solution of equation 2 involves replacing the derivatives dC/dz, $d^2C/dz^2$, by finite difference forms. For space derivatives, the central difference approximations (Gerald [15]) were used as follows:

$$\frac{dC_Z(K)}{dz}=\frac{C_Z(K+1)-C_Z(K-1)}{2(\Delta z)} \tag{3}$$

$$\frac{d^2C_Z(K)}{dz^2}=\frac{C_Z(K+1)-2C_Z(K)+C_Z(K-1)}{(\Delta z)^2} \tag{4}$$

To solve set of non-linear differential equations, D02EBF Nag Fortran Library Routine was used. Peclet number for each recirculating pump rate was estimated using parameter estimation program, E04VDF Nag Fortran Library Routine. This procedure requires Peclet number to be changed until the numerical prediction of the model and the experiments agree with some criterion of best fit. The estimated value gives the minimum variance of the experimental points about the theoretical function.

## 3. MATERIALS AND METHODS

### *3.1 Experimental Apparatus:*

The mixing experiments were carried out in a combined two reactors system, pyrex glass fermenter (CSTR) with a working volume of 2.0 L and a loop of helical glass pipe (PFTR), 0.7 cm in diameter (i.d.) and 870 cm long with helical diameter of 29 cm, with a working volume 0.5 L. To approach more closely to plug flow[13-14], a long and narrow coiled tube was used as the apparatus. The input of the loop was fixed into the bottom plate of the stirred tank fermenter by a rubber tubing (0.8 cm in diameter (i.d.)) via bubble breaker to prevent the air bubbles passing through the loop and a peristaltic pump for recycling the fermenter liquid. The output of the loop was fixed to the top plate of the stirred tank fermenter by a rubber tubing. The output of the loop was also provided with a sample port. Whole assembly (PFTR) was placed into a temperature controlled water bath to ensure that the distilled water was kept at constant temperature throughout the system. The glass loop together with the rubber tubing was designed to have 0.5 litre of capacity. Feeding rate (f) to CSTR was set at 0.66 L/h.

### 3.2 Operating conditions:

To describe mixing in the tubular section of the system, the recirculating pump rate in this part of the system was varied from 1.5 L/h to 60.0 L/h with different intervals. The aeration rate supplied only for the first reactor (CSTR) of the model system was maintained at 1.5 L/min. The impeller speed was set at 300 rpm (temperature, $37\mp0.5$ $^0$C and pH, $7\mp0.5$). These conditions were planned to use later in the combined two reactors fermentation (CSTR+PFTR) system to determine the effect of imperfect mixing on the performance of the *Escherichia coli* K12.

### 3.3 Determination of methylene blue concentration:

During the dye experiments, a 5.0 mL solution containing 17.6 mg methylene blue was added quickly to the CSTR using a BCL autopipette through a port in the top plate. Samples of approximately 3 mL were collected at regular time intervals from the outlet of the both of the reactors (CSTR's and PFTR's sampling ports). Methylene blue concentrations were found by measuring the absorbance of the samples taken from the output of the both reactors. The absorbance of the samples were measured on a Pye Unicam PS8 400 (uv/vis) double beam spectrophotometer in the plastic cuvettes of 1.0 cm path length at a wavelength of 660 nm.

**4. RESULTS AND DISCUSSIONS**

Six runs were carried out to determine the Peclet numbers. The experimental data were then used in the mathematical model (equations 1 and 2) to optimise the Peclet numbers using the parameter optimisation routine E04VDF from Nag library. The experimental and theoretical dye (methylene blue) concentration values were plotted as a function of time in Figures 2-7 for the recirculating pump rates of 1.5 L/h, 3.0 L/h, 6.0 L/h, 15.0 L/h, 30.0 L/h and 60.0 L/h, respectively. The close agreement between the experimental data sets and the theoretical data sets can clearly be seen in these figures.

As it is seen in Table 1, the Peclet numbers fluctuate with the recirculating pump rates. Reynolds numbers less than 2000 (in the laminar flow), Peclet numbers are decreasing from 47.29 to 5.13 and dispersion increases. Because, Peclet numbers are dependent on Schmidt (Sc) number or molecular diffusivity in the laminar flow regimes (Wen [14]). When, Reynolds number higher than 2000 (in the turbulent flow), Peclet numbers are increasing from 270 to 700. The increase in the Peclet numbers is affected by the increasing value of the eddy diffusivity of mass ($e_D$), as turbulence is being generated (Wen [14]).

Another approach was also considered to calculate the Peclet numbers using equation 5 of Taylor (Wen [14]).

$$\frac{1}{Pe}=\left(\frac{3.10^7}{Re^{2.1}}+\frac{1.35}{Re^{1/8}}\right)\frac{d_t}{L} \qquad (5)$$

The Peclet numbers for the recirculating pump rates of 30.0 L/h and 60.0 L/h were then calculated and the Peclet numbers of 404 and 1250 were found for these pump rates, respectively. After using the parameter estimation program, the corresponding Peclet numbers of 270 and 700 were found, which are somewhat smaller than the ones calculated from the equation mentioned above. The reason for this could be the coiled tubular reactor which was used in this study rather than the linear tube, which was used to establish the correlation equation. It is also known that the axial mixing increases in the coiled tubular reactor(Bischoff [16]). Peclet numbers depend on the shape of the tubular loop, helical diameter of the loop, curve angle of the loop, diameter of the loop, friction of the loop material and the specifications of the liquid used in the loop, etc. Therefore, the estimated Peclet numbers are considered to be realistic values and it was decided to use later in the mathematical modeling of the combined two reactors system considering the microorganism.

**Table 1.** Results from the parameter estimation program for each recirculating pump rate

| Recirculating pump rates, L/h | 1.5 | 3.0 | 6.0 | 15.0 | 30.0 | 60.0 |
|---|---|---|---|---|---|---|
| Reynolds numbers | 101 | 202 | 404 | 1010 | 2020 | 4040 |
| Starting values of the Peclet numbers | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Estimated values of the Peclet numbers | 47.3 | 26.9 | 12.9 | 5.1 | 270.0 | 700.0 |
| Objective function values from the starting values, ($F_S$) | 64.0 | 58.1 | 33.8 | 35.2 | 53.5 | 50.9 |
| Objective function values from the estimated values, ($F_E$) | 23.6 | 22.7 | 9.2 | 20.7 | 20.4 | 18.3 |
| Percentage values of the improvements | 63 | 61 | 73 | 41 | 62 | 64 |

In Table 1, objective function values from starting values and from the estimated values ($F_S$ and $F_E$) were calculated by using the equation given below;

$F_i = ABS\,[F(X_{exm.}) - (X_{theo.})]$

$_I$ represents $_S$ or $_E$

$_S$ represents the objective function values calculated by using the starting values

$_E$ represents the objective function values calculated by using the estimated values

$F(X_{exm.})$ represents the experimental value of the function

$F(X_{theo.})$ represents the theoretical value of the function

Then, percentage values of the improvements were calculated by using the equation given below;

Percentage values of the improvements = $100.[1- (F_{E.}/F_{S.})]$

Although, there is no work directly related to the present study, Purgstaller [9] simulated the combine system of STR+PFTR using temperature profile method. They developed mathematical equations to simulate the system for all loops. Their results showed some deviations between the computer simulation and the experimental results due to the neglected backmixing in the PFTR section of the system.

Toda [7] also studied the combine system of CSTR+PFTR by computer simulation without verifying it experimentally. Their aim was to show that steady-state continuous culture is possible at dilution rates higher than the maximum specific growth rate of microorganisms. They set the axial mixing in the tubular-loop fermenter at an intermediate value of Pe=3.0 between complete mixing and plug flow for every recirculating pump rates. However, Pe=3.0 was also used for every recirculating pump rates in the mathematical model of the present study and it was found that the theoretical dye concentration values were deviated from the experimental values. Then Peclet numbers for each recirculating pump rate were estimated using Pe=3.0 as the starting value in the optimisation program. These results are also shown in Table 1. The objective functions were also included in this table to show that an improvement of up to 73% could be achieved with respect to the objective function with Pe=3.0. Thus, as shown in Figures 2-7 very good agreement were obtained between theoretical and experimental results.

To sum up, in this work, a model bioreactor for mixing studies was presented and its physical behaviour investigated. In addition to the use of this model reactors for investigating mixing, they can be used at the same time for elucidating the influence of oxygen on bioprocessing. Thereby, compartment 1 is aerated and the second region is deaerated, so that the mean residence time t of cells in both regions can be varied. Another parameter is the circulation time distribution CTD, which is different in a stirred tank and a plug flow compartment. By using mixing parameters in the mathematical models, the effect of limited parameters could be investigated. For example, the different dissolved oxygen gradients will partly be imposed on the microorganisms as a result of the inadequate mixing and partly be a result of the microorganism's own metabolic activity. Thus, the cells will experience a changing macro as well as micro environment. This may give rise to physiological variations which will be accentuated as the circulation loop increases within the fermenter volume.

## 5. CONCLUSIONS

A tubular loop reactor connected to a continuous stirred tank reactor has been designed with view to simulate the circulation loops found in the large-scale stirred tank reactors. It appeared that this system can be used to simulate large-scale stirred tanks, thus providing a cheap method for studies on scale-up. The gradients in stirred tanks set up as a result of poor mixing are well demonstrated in a combined two reactors system (CSTR + PFTR).

The mixing performance of the plug flow reactor of the combined system was investigated by determining the Peclet numbers for various recirculating pump rates. These were theoretically estimated by using the results obtained from the experiments. To determine the degree of backmixing, Peclet numbers need to be known. For the recirculating pump rates of 15 L/h (laminar flow regime) and 60.0 L/h (turbulent flow regime), minimum Peclet number of 5.1 (maximum axial dispersion) and maximum Peclet number of 700 (minimum axial dispersion) were obtained, respectively. It can be seen that as $1/Pe \rightarrow 0$, plug flow is obtained and as $1/Pe \rightarrow \infty$, the flow approaches the well mixed situation. These estimated Peclet numbers could be used in modeling of the expression of enzymes and the metabolic end product formation associated with the growth of microorganism in variable environmental conditions (including oxygen and substrate limitation) for the same process conditions.

## 6. NOMENCLATURE

$C_B$, exit concentration of material from the CSTR, $\mu$mole/L
$C_Z$, exit concentration of material from the PFTR, $\mu$mole/L

K, integer; 1,2,....,N; N=50
$d_t$, diameter, m
$E_Z$, axial dispersion coefficient, $m^2/h$
f, feeding flow rate, L/min
fr, flow rate of recirculating pump, L/min
L, total length of the PFTR, m
Pe, Peclet number, $U.z_e/E_Z$
Re, Reynolds number
t, time, h
U, velocity, m/h
$V_B$, volume of the CSTR, L
z, length of the PFTR, m
$z_e$, total length of the PFTR, m

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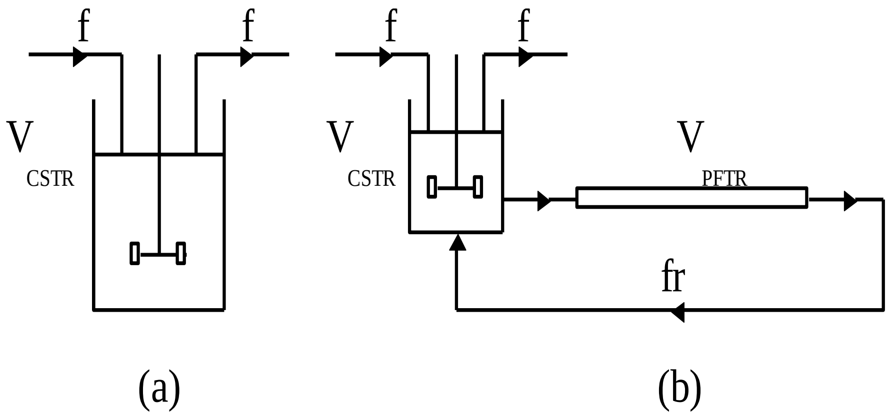


**Figure 1.** (a) Continuous fermentation (CSTR) in large-scale, (b) simulation of the model bioreactor system in small-scale