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Engineering approach to mixing quantification in bioreactors

B. Mayr, Graz, Austria, **P. Horvat,** Zagreb, Croatia and **A. Moser,** Graz, Austria

Abstract. "Homogeneity-time" is defined and introduced as the criterion for mixing quality in bioreactors. The criterion could replace the mixing time, in the case, when more than one measuring point (sensors) is included in the measuring system. Results based on the homogeneity-time and the temperature pulse method, achieved in stirred tank reactors under aerated conditions as well as in a jet-mixed tank, are presented.

List of symbols

$c_{P,P}$	kJ/kg K	Heat capacity of the pulse medium
$c_{P,S}$	kJ/kg K	Heat capacity of the reactor-medium
F	m^3/s	Flow rate of the pulse-input
i	–	Inhomogeneity
I_N	–	Inhomogeneity-number
$M(t)$	°C	Ideal response curve
m	–	Number of combinations for certain number of sensors acc. to Table 1
n	–	Number of sensor
ϱ_P	kg/m^3	Density of the pulse medium
ϱ	kg/m^3	Density of the tank medium
s_1	°C	Mean absolute deviation of the sensor temperatures related on the ideal response curve
s_2	s	Mean absolute deviation of the homogeneity-times related on the time achieved with 6 sensors
t	s	Time
$t(i)$	s	Homogeneity-time
t_{PS}	s	Starting time of tracer injection
t_{PE}	s	End time of tracer injection
T_E	°C	Mean medium temperature at the end of experiment
T_k	°C	Temperature at k-th sensor position
T_P	°C	Pulse temperature
T_S	°C	Mean medium temperature before the tracer injection
V_S	m^3	Tank volume before pulse input

1 Introduction

Mixing time, primarily intended to quantify the mixing behaviour of discontinuous stirred tank reactors is widely introduced as the criterion for mixing quality in all other discontinuous types of bioreactors. In the literature two different, but similar, definitions of mixing time based on the degree of mixing [1] can be found: The first one defines the mixing time as the amount of time necessary to reach the particular degree of mixing [2]; the second one, as the mean value of two timepoints characterised by the intersection of the envelope of curves with decaying amplitude and straight lines which represents the degree of mixing [3]. The first method is useful if the response function contains a low number of minima and maxima (fast homogenisation in the system) and the second one should be applied if there are several minima and maxima in the response function (slow homogenisation). In the case, when the experiments are carried out using more than one sensor, in the way it was done in the study of mixing properties of air-lift reactors and bubble columns [4], the number of achieved mixing times corresponds to the number of sensors. In their work [4] the mixing time was chosen to be the average value of all mixing times achieved. The occurrence of different mixing times, by using several sensors positioned in the bioreactor is an indicator for an inhomogeneity in the whole system. The inhomogeneity of a reactor was introduced by Danckwerts [5], as the "degree of segregation" for continuous operating systems, and it was further developed by Zwietering [6] as the "maximum mixedness". Furthermore, the inhomogeneity model was used for growth modelling [7] and to investigate loop-reactors [8, 9]. The aim of this work is to apply "inhomogeneity" to examine the overall mixing properties of different bioreactors operating batchwise as well as to determine the influence of number and position of sensors on the quality of the resulting homogeneity-time.

2 Materials and methods

2.1 Bioreactors

In our experimental work bioreactors with the following technical equipment were used:

a) Bioreactor supplied with one Rushton turbine, 3.2 m^3 of total volume, tank diameter 1.18 m, power consumption

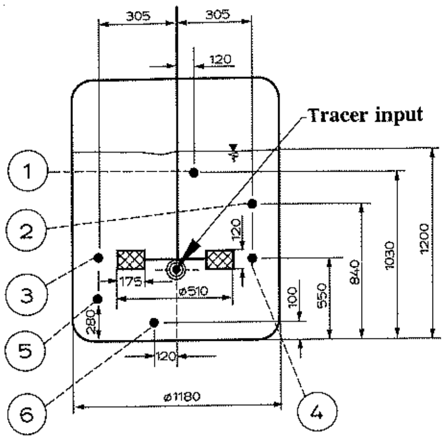

Fig. 1. Experimental setup for stirred tank with one Rushton turbine indicating the positions of tracer input and sensors (No. 1–6)

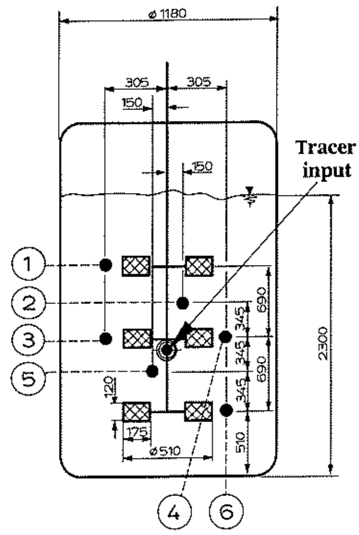

Fig. 2. Test setup for stirred tank with three Rushton turbines

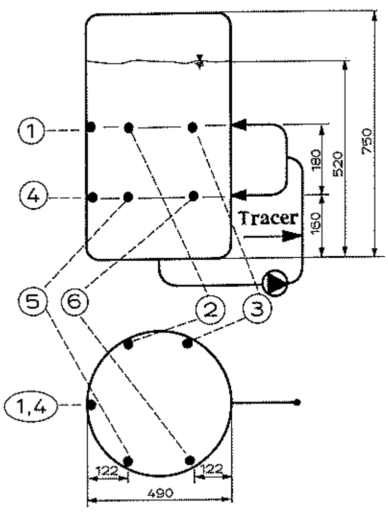

Fig. 3. Experimental arrangement in jet mixed bioreactor

max. 26 kW, turbine diameter 0.51 m, liquid volume 1.2 m^3, 4 baffles.

b) Bioreactor supplied with three Rushton turbines, 3.2 m^3 of total volume, tank diameter 1.18 m, power consumption max. 26 kW, turbine diameter 0.51 m, liquid volume 2.5 m^3, 4 baffles.

c) Bioreactor (INSA Toulouse) supplied with a recirculation-system consisting of two jets and one pump, 0.9 m^3 of total volume, tank diameter 0.49 m, power consumption max. 1.0 kW [10, 11].

2.2 Measuring equipment and conditions

In our experiments the temperature pulse method and hot water (95 °C) as tracer were used [4, 12]. The data aquisition system is equipped with six Pt-100 sensors (t_{90} response time 0.08 s, resolution 0.002 °C) coupled with a data-logger unit (Fa. PAAR Graz, Austria) and personal computer. The sensor positions in the bioreactors as well as the spot of the pulse input are shown in Figs. 1–3.

In the stirred tank supplied with one turbine the locations of the sensors were chosen in the following manner: two sensors are positioned close to the turbine in order to record a reliable signal for the well mixed compartment; two of the sensors are put in the upper circulation loop so that they are situated after each third of longest possible path [13] with necessary distance to the wall and to the surface; the remaining two sensors are put in the same way in the lower circulation loop.

Concerning the stirred tank equipped with three turbines sensor positions were defined as follows: Two sensors are arranged close to the middle turbine in order to record mixing in the compartment of pulse injection; two sensors were installed close to the upper and lower stirrer to quantify the mixing in the other two well mixed compartments; the last two sensors were located in the transient regions between the stirrers.

In the jet-mixed tank two different circulation paths can be observed. Therefore three of the sensors were ordered in each path, one opposite to the jet entrance (micromixer), the others after each third of the backward path from first sensor to the jet entrance (macromixer).

The pulse-input-time was 1 second for the stirred tanks and 2 seconds for the jet bioreactor. The pulse was injected in a highly turbulent part of the reactors in order to be able to trace its path.

All experiments with the given stirred tanks have been carried out in air-water system in the range of the following conditions: Turbine rotation 0–200 rpm, aeration rate 0–250 m^3/h.

All experiments with the jet-mixed tank have been carried out in a water system in the range of a pump capacity 0.15–0.8 m^3/h.

3 Theory

The temperature pulse imposed on the bioreactor leads to an increase in temperature in the whole system. In the case of an ideally well mixed system the response function to the tracer input is defined by the following mathematical expressions:

$$M(t) = T_S\,, \qquad t < t_{PS} \tag{1}$$

$$M(t) = f(t)\,, \qquad t_{PS} < t < t_{PE} \tag{2}$$

$$M(t) = T_E\,, \qquad t > t_{PE}\,. \tag{3}$$

In the experiment the temperature of the tracer (T_P) and the time independent volumetric flow rate (F) by the injection in the system is strictly defined, as well as the time interval of this flow ($t_{PE} - t_{PS}$). Therefore it is easy (for the perfectly mixed reactor) to define the function $f(t)$ for the transient state during tracer injection:

$$f(t) = \frac{C_{P,P}}{C_{P,S}}\, T_P - \frac{\dfrac{C_{P,P}}{C_{P,S}}\, T_P - T_S}{\left[1 + \dfrac{F}{V_S}\, t\right]^{\varrho_P/\varrho}}\,. \tag{4}$$

Observing these preconditions it is possible to use the ideal response function $M(t)$ as the basis for the definition of the "inhomogeneity" (i) used here.

The mean absolute deviation for the whole reactor with the applied measuring system is defined in the following way:

$$s_1(t) = \frac{1}{n} \sum_{k=1}^{n} |T_k - M(t)|\,. \tag{5}$$

It is important to point out that it is not possible to use the standard deviation (s_1), because the sensor-points are well defined and not normally distributed.

The mean absolute deviation is dependent on the temperature increase ($T_E - T_S$). This increase, caused by the hot pulse, depends on pulse quantity and temperature as well as on volume and temperature in the vessel. Therefore a dimensionless standardization in the following way is applied to the curves in order to be able to compare the results:

$$i(t) = \frac{s_1(t)}{T_E - T_S}\,. \tag{6}$$

This equation represents the time dependency of inhomogeneity for the whole reactor system if all significant compartments in the reactor are measured. Based on Eq. (6) it is possible to define the "homogeneity-time" as the time elapsed from the pulse injection until the inhomogeneity function $i(t)$ drops below a certain conventional value. The homogeneity-time defined in this way could perhaps replace the widely used mixing time as a criterion for the mixing property of bioreactors if more than one sensor is used.

As a second possibility to discribe the inhomogeneity in the reactor, the "inhomogeneity-number" (I_N) as the mean value of inhomogeneity of the entire reactor volume for the defined time period might be useful:

$$I_N = \frac{1}{t - t_{PS}} \int_{t_{PS}}^{t} i(t)\, dt\,. \tag{7}$$

According to Eq. (7) the inhomogeneity-number in the whole reactor is proportional to the area under the inhomogeneity curve and is reversely proportional to the time interval of integration. In order to evaluate this area in the experimental work, it is necessary to use a mathematical method of numerical integration. This integration must be carried out until some certain predefined level of the inhomogeneity-number is reached. The solution of this problem can only be obtained if an iterative procedure is utilized. Since it is not possible to calculate the inhomogeneity-number without knowing the inhomogeneity curve and since both mentioned criterions (homogeneity-time or inhomogeneity-number) make use of conventional values, we focused our attention on the evaluation of the homogeneity-time.

4 Results and discussion

Typical results are chosen to demonstrate the procedure of calculating the inhomogeneity. In Figs. 4–6 system responses of the mentioned bioreactors are illustrated. From the Figs. 4–6 it is obvious that according to the method described earlier [2] six values for mixing times are achieved. In other words: Each result depends strongly on the measuring point. The "point" is defined as a "small" volume around the sensor [5].

In earlier experiments with a measuring system containing more than one sensor that are referred to in the literature [4], the arithmetic mean value of the mixing times of the individual sensors was used as the characteristic mixing time for the whole bioreactor. We assumed that the differences between mixing times represent the inhomogeneity of the bioreactor. In order to describe the state of mixing in the whole tank the definition of the inhomogeneity according to Eq. (6) was established. Results of this procedure, shown in the Figs. 7–9, correspond to the same experiments illustrated in Figs. 4–6.

The mixing time is the elapsed time until a predefined degree of mixing, e.g. 85%, is achieved. In the proposed method the homogeneity-time is defined as the time elapsed until the inhomogeneity curve of the system (according to Eq. (6)) declines to a certain level, e.g. 0.15 (Figs. 7–9). Because of the above definition, the inhomogeneity is only a linear transformation of sensor signals. Therefore a inhomogeneity value of 0.15 corresponds to a mixing degree of 85%.

Since mixing time and homogeneity-time are related to each other, the influence of the position and the number of sensors appears in both cases. In order to evaluate this influence, a test of the "inhomogeneity method" was performed using all possible combinations of positions (Figs. 1–3) with different number of sensors (1–6) according to Table 1. The

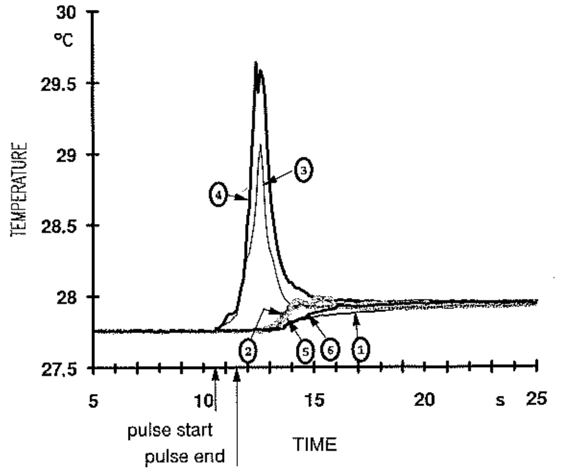

Fig. 4. System response of stirred tank with one Rushton turbine to temperature pulse, sensor positions acc. to Fig. 1. Experimental conditions: Aeration 40 m^3/h, stirrer speed 100 rpm

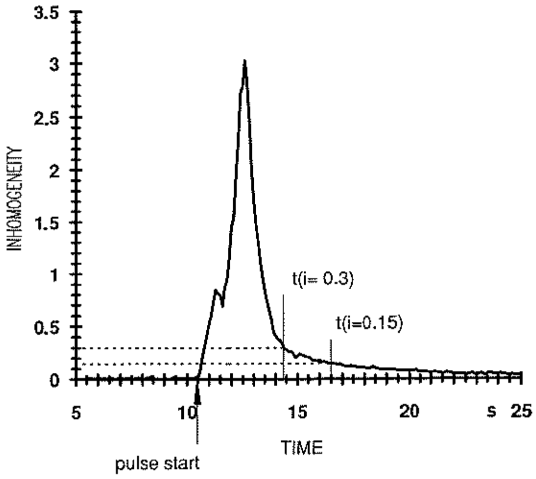

Fig. 7. Inhomogeneity curve and resulting homogeneity-times calculated from system response shown in Fig. 4

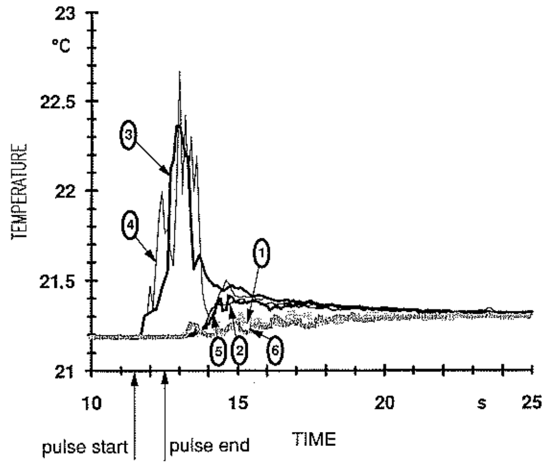

Fig. 5. System response of stirred tank system with three Rushton turbines to temperature pulse, sensor positions acc. to Fig. 2. Experimental conditions: Aeration 40 m^3/h, stirrer speed 100 rpm

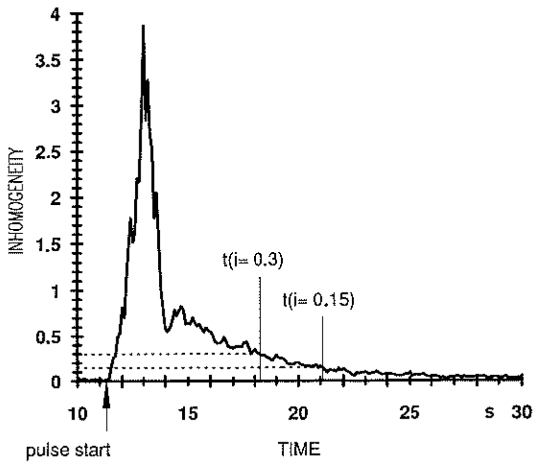

Fig. 8. Inhomogeneity curve and homogeneity-times obtained from system response illustrated in Fig. 5

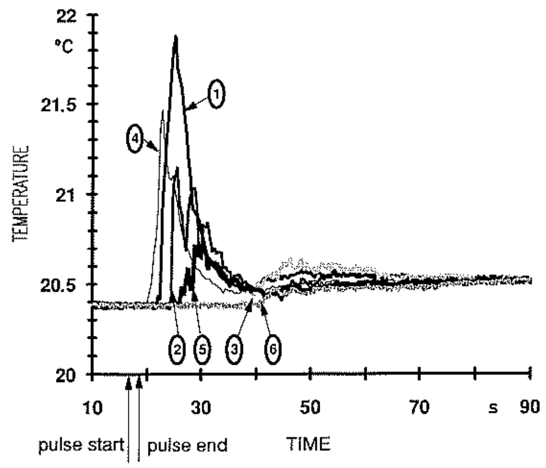

Fig. 6. System response of jet mixed reactor to temperature pulse, sensor positions acc. to Fig. 3. Experimental conditions: Pump capacity 0.4 m^3/h

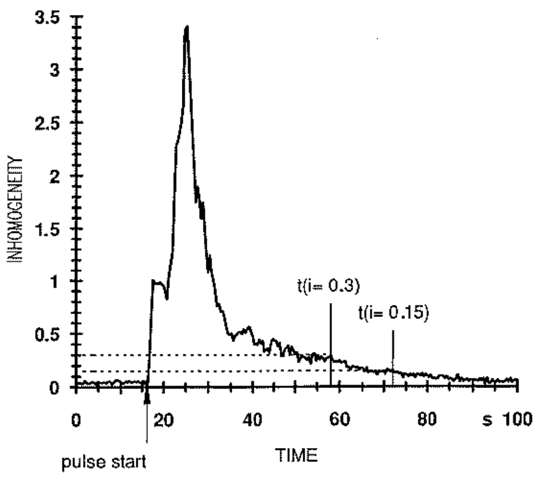

Fig. 9. Inhomogeneity curve and homogeneity-times achieved from system response according to Fig. 6

Table 1. List of combinations of different sensor numbers placed in six positions (Figs. 1–3)

Combination	Number of sensors used	Position included in combination					
1	6	1	2	3	4	5	6
2	5	–	2	3	4	5	6
3	5	1	–	3	4	5	6
4	5	1	2	–	4	5	6
5	5	1	2	3	–	5	6
6	5	1	2	3	4	–	6
7	5	1	2	3	4	5	–
8	4	–	–	3	4	5	6
9	4	–	2	–	4	5	6
10	4	–	2	3	–	5	6
11	4	–	2	3	4	–	6
12	4	–	2	3	4	5	–
13	4	1	–	–	4	5	6
14	4	1	–	3	–	5	6
15	4	1	–	3	4	–	6
16	4	1	–	3	4	5	–
17	4	1	2	–	–	5	6
18	4	1	2	–	4	–	6
19	4	1	2	–	4	5	–
20	4	1	2	3	–	–	6
21	4	1	2	3	–	5	–
22	4	1	2	3	4	–	–
23	3	–	–	–	4	5	6
24	3	–	–	3	–	5	6
25	3	–	–	3	4	–	6
26	3	–	–	3	4	5	–
27	3	–	2	–	–	5	6
28	3	–	2	–	4	–	6
29	3	–	2	–	4	5	–
30	3	–	2	3	–	–	6
31	3	–	2	3	–	5	–
32	3	–	2	3	4	–	–
33	3	1	–	–	–	5	6
34	3	1	–	–	4	–	6
35	3	1	–	–	4	5	–
36	3	1	–	3	–	–	6
37	3	1	–	3	–	5	–
38	3	1	–	3	4	–	–
39	3	1	2	–	–	–	6
40	3	1	2	–	–	5	–
41	3	1	2	–	4	–	–
42	3	1	2	3	–	–	–
43	2	–	–	–	–	5	6
44	2	–	–	–	4	–	6
45	2	–	–	–	4	5	–
46	2	–	–	3	–	–	6
47	2	–	–	3	–	5	–
48	2	–	–	3	4	–	–
49	2	–	2	–	–	–	6
50	2	–	2	–	–	5	–
51	2	–	2	–	4	–	–
52	2	–	2	3	–	–	–
53	2	1	–	–	–	–	6
54	2	1	–	–	–	5	–
55	2	1	–	–	4	–	–
56	2	1	–	3	–	–	–
57	2	1	2	–	–	–	–
58	1	1	–	–	–	–	–
59	1	–	2	–	–	–	–
60	1	–	–	3	–	–	–
61	1	–	–	–	4	–	–
62	1	–	–	–	–	5	–
63	1	–	–	–	–	–	6

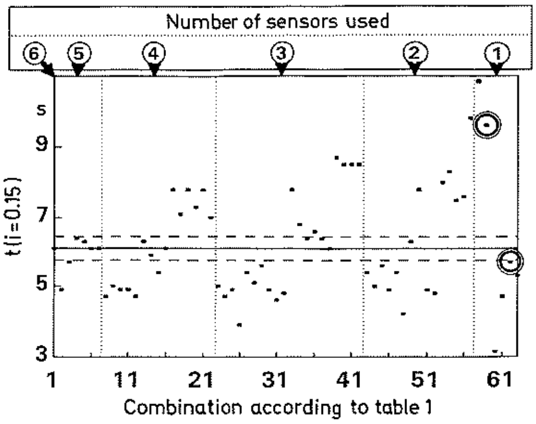

Fig. 10. Homogeneity-times achieved with combinations (number and position) of sensors according to Table 1. Stirred tank with one Rushton turbine, same conditions as in Fig. 4. Details explained in text

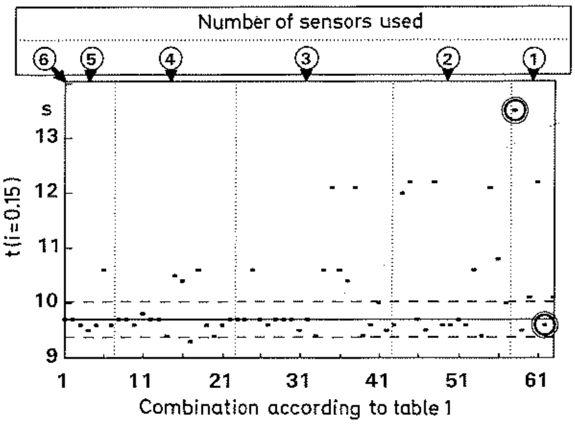

Fig. 11. Homogeneity-times obtained with sensor combinations (number and position) according to Table 1. Stirred tank with three Rushton turbines, conditions see Fig. 5

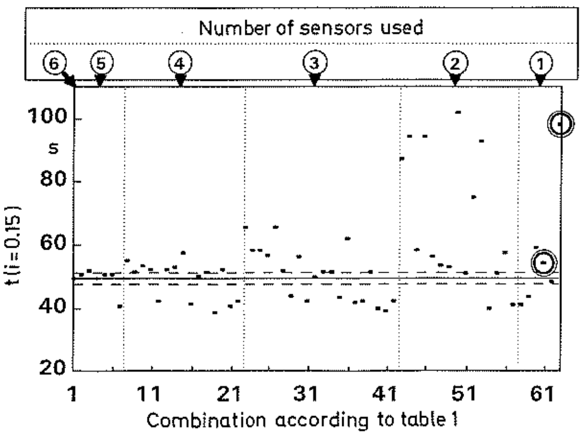

Fig. 12. Homogeneity-times resulted from sensor combinations (number and position) according to Table 1. Jet mixed tank, conditions see Fig. 6

calculated homogeneity-times (for degree of 0.15) are illustrated in the Figs. 10–12 according to experimental conditions listed in Table 1.

It is obvious from Figs. 10–12 that the scatter of the results for homogeneity-times increases with decreasing number of sensors. The deviations by using five sensors is significantly lower than with a lower number of sensors. Therefore, the result with six sensors was used as the reference for further calculations. This is indicated in Figs. 10–12 by a solid horizontal line. The dashed lines in these figures represent the mean absolute deviation of the results achieved by using five sensors at six different positions and is defined in the following way:

$$s_{2,n} = \frac{1}{m} \sum_{j=1}^{m} |t(i)_{n,j} - t(i)_6| \quad (8)$$

The percentage of the deviations for the experiments with 1 to 5 sensors related to the time achieved with six sensors is documented in Fig. 13.

In order to be able to quantify the minimum amount and the best point of the sensors to show approximately the same result as with six sensors, the range between the dashed lines in Figs. 10–12 is used to detect representativ combinations of sensors. From results included in Fig. 13 the following conclusions may be drawn:

a) For the examined stirred tank with three Rushton turbines already two sensors lead to results that are in the range of 10% deviation from the values achieved with six sensors;

b) for the examined stirred tank with one Rushton turbine five sensors must be used to get a result of the same quality;

c) for the tested jet mixed tank under same criterion four sensors are sufficient.

The next step was to examine which sensor positions are significant for the mixing behaviour of the whole tank. Therefore the combinations of sensors which show a result in the range between the two dashed lines on Figs. 10–12 were analyzed. The frequencies of the various positions are listed in Table 2.

Two positions with the highest frequencies (sensors No.1 and 5 for tank with one Rushton turbine, sensors No. 2 and 5 for tank with three Rushton turbines, sensors No. 6 and 4 for jet mixed tank) were investigated if they, being used alone, give an homogeneity-time which is in the range between the dashed lines in Figs. 10–12. These points are marked with circles on the mentioned figures. It turns out that, if the positions with the highest frequencies are analzyed, the homogeneity-times related to this sensor position, are not always in the "good" range.

Therefore it can be strictly concluded that there is no single sensor position describing the whole tank with high reliability, because the fluctuations of results compared to homogeneity-times achieved with six sensors are in the range of 38%, 12.8% and 41% for the stirred tank with one Rushton turbine, stirred tank with three Rushton turbines and jet-mixed tank respectively.

The discussed method of inhomogeneity was used to investigate the mixing properties in bioreactors under the conditions described in the section on materials and methods. The results are presented in Figs. 14–16.

Since the inhomogeneity curve described the mixing behaviour of the whole reactor if a large enough number of

Table 2. Frequencies of homogeneity times in the range of mean absolute deviation obtained by using five sensors in six different positions

Reactor	Sensor positions					
	1	2	3	4	5	6
With 1 turbine	11	25	20	18	24	18
With 3 turbines	11	6	8	8	9	7
Jet mixed	13	13	8	14	9	16

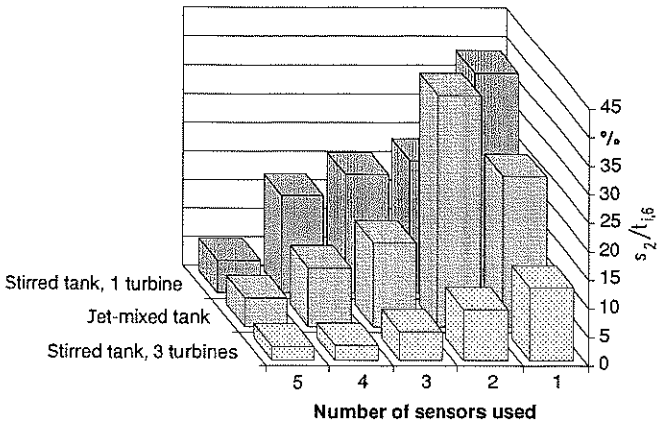

Fig. 13. Influence of sensor number on percentage of deviations (s_2) related to the homogeneity-time achieved with six sensors in different bioreactors

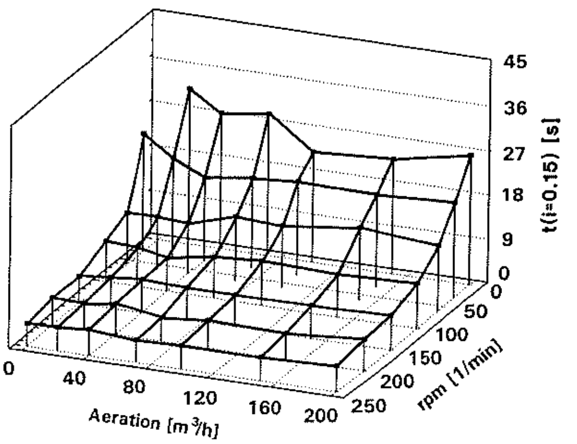

Fig. 14. Dependence of homogeneity-time ($i = 0.15$) on aeration rate and stirrer rotation for stirred tank with one Rushton turbine

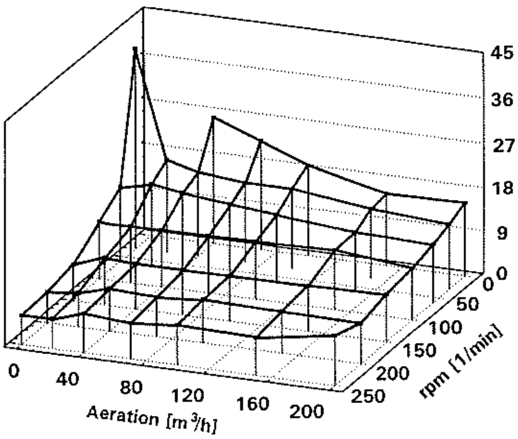

Fig. 15. Dependence of homogeneity-time ($i = 0.15$) on aeration rate and stirrer rotation for stirred tank with three Rushton turbines

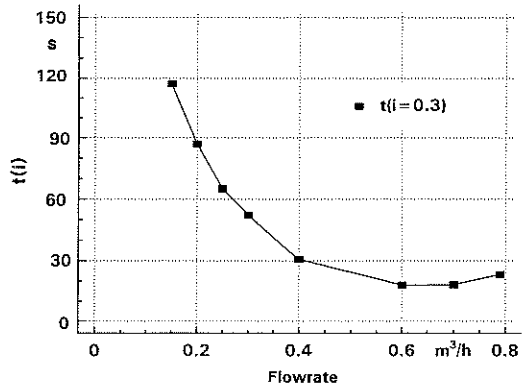

Fig. 16. Dependence of homogeneity-time ($i = 0.3$) on pump capacity for jet mixed tank

sensors is applied, it is a useful and exact tool as a criterion for the parameter optimisation process in macroscopic mathematical modelling of mixing in bioreactors. Future work should clarify the significance of this approach for bioprocessing based on analysis of the inhomogeneity curve.

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B. Mayr
A. Moser (corresponding author)
Institute for Biotechnology
Petersgasse 12
8010 Graz
Austria

P. Horvat
Faculty of Food Technology and Biotechnology
University of Zagreb
Pierottijeva 6
41000 Zagreb
Croatia