Performance determination of a single cell oil process.

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Abstract

The development of a process is submitted to a necessary and essential phase of optimization to use it in the best way. Experimental design allows to obtain a maximum of information with a minimun of experiments. A new approach based on fuzzy logic, named Fuzzy Dynamic Experimental Design (F.D.E.D.), developed in our laboratory, has been validated on a bioprocess. The interest of this work is to show the efficiency of this method on a chosen example: the production of single cell oil. This study comes within already realised works on a yeast: Yarrowia lipolytica*. With the help of F.D.E.D., the covering rate of the domain has been evaluated. Then, we had to add few experiments to increase the domain covering. The validity of the calculated models has been verified with new experiments not used for the identification of parameters. Results show a satisfactory prediction.*

1. Introduction

F.D.E.D. is a new method of experimental planning developped within the L.S.G.C.. This one has already been successfully tested on chemical reactions and especially on the decylation of lactose (Fonteix *et al.*, 1997).

Characteristics of this method justify to test it on a bioprocess for many reasons. First, more than chemical reactions, bioprocesses are often dynamic phenomena characterized by strongly non linear variations. These characteristics restrict the use of classic planning well suited for static experiments and for models which are non linear on their parameters. Thus, it is very difficult to choose a model prior to experimentation what is essential in the case of the use of classical method. This is difficult without knowledge or with partial one. For this reason, a method, like F.D.E.D., when choice of the model *a priori* is not necessary, is interesting to realise initial experiments or to complete previous ones. Besides, implemented processes are often costly in time and experiments. Then F.D.E.D. is able to use all available knowledge by integrating it in the design. We can employ an evolutive method, with no loss of information, and, besides, which allows to complete the information in an iterative way.

Then, F.D.E.D. seems to answer correctly to these constraints but has not been tested yet on a bioprocess. Consequently, we have chosen to apply it to the production of single cell oil and especially to obtain a cocoa butter equivalent in a microbial way.

2. F.D.E.D. theory

2.1. References definition

Classical experimental design defines a set of "static" experiments $\mathbf{E} = \{E_1, E_2, ... E_n\}$. Here, experiments E_i are references which are not realized. Suppose that a dynamic experiment is made with sampling time t_k (k varies from 1 to p).

"Static" experiment E_i is a set of qualification levels corresponding to each measured state variable x_j (j varies from 1 to q): $E_i = \{L_{i1}, L_{i2}, ... L_{ij}, ... L_{iq}\}$.

Reference fuzzy set F_{ik} of E_i, $F_{ik} = \sum_{Lij \in Ei} f_{ijk}/L_{ij}$, represents the total accomplishment of experiment E_i at sampling time t_k. Thus $f_{ijk} = 1 \ \forall$ i, j, k.

2.2. Experimental data treatment

2.2.1. Expression of r_i

The dynamic experiment is realized. So, the fuzzy set V of **E**, $V = \sum_{Ei \in \mathbf{E}} r_i/E_i$, is the accomplishment of the experimental design through the dynamic experiment. But $V = \cup_{(k)} V_k$ with $V_k = \sum_{Ei \in \mathbf{E}} a_{ik}/E_i$, the accomplishment of the experimental design through the dynamic experiment at sampling time t_k.

Then it results: $r_i = \sup_{(k)} (a_{ik})$.

2.2.2. Expression of a_{ik}

Fuzzy set W_{ik} of E_i, $W_{ik} = \sum_{Lij \in Ei} b_{ijk}/L_{ij}$, is the accomplishment of the reference through the dynamic experiment. So, b_{ijk} is the possibility that the experimental measurement of variable x_j at time t_k is compatible with the qualification level L_{ij}. Thus, a_{ik} is the necessity of W_{ik} referring to F_{ik}.

It reads: $a_{ik} = N(W_{ik} ; F_{ik}) = \inf_{(Lij \in Ei)} \max(b_{ijk} , 1-f_{ijk}) = \inf_{(Lij \in Ei)} b_{ijk}$.

2.2.3. Expression of b_{ijk}

Membership function $\mu_{Ajk} (x_j)$ of fuzzy set A_{jk}, defined by $A_{jk} = \int_{xj} \mu_{Ajk} (x_j)/x_j$, is deduced from measurement and simulation of x_j at t_k. This membership function integrates known measurement uncertainty and model accuracy. Membership function $\mu_{Lij} (x_j)$ of fuzzy set Q_{ij}, defined by $Q_{ij} = \int_{xj} \mu_{Lij} (x_j)/x_j$, is associated to each qualification level L_{ij}. Human expert chooses qualification levels and associated membership functions. Thus, b_{ijk} is the possibility of A_{jk} referring to Q_{ij} and $b_{ijk} = \Pi(A_{jk} ; Q_{ij}) = \sup_{(xj)} \min(\mu_{Ajk} (x_j) , \mu_{Lij} (x_j))$.

2.2.4. Membership function μ_{Ajk}

Membership function $\mu_{mjk}(x_j)$ of fuzzy set M_{jk}, defined by $M_{jk} = \int_{xj} \mu_{mjk}(x_j)/x_j$, is deduced from measurement of x_j at t_k and its known uncertainty (figure 1a). This membership function can be triangular or gaussian. μ_{mjk} is the possibility to have x_j at t_k according to measurement at this time. Membership function $\mu_{sjk}(x_j)$ of fuzzy set S_{jk}, defined by $S_{jk} = \int_{xj} \mu_{sjk}(x_j)/x_j$, results from simulation of x_j at t_k and from the acceptable inaccuracy of the model, chosen by the human expert (figure 1b). Thus $A_{jk} = M_{jk} \cap S_{jk}$ and $\mu_{Ajk}(x_j) = \min(\mu_{mjk}(x_j), \mu_{sjk}(x_j))$.

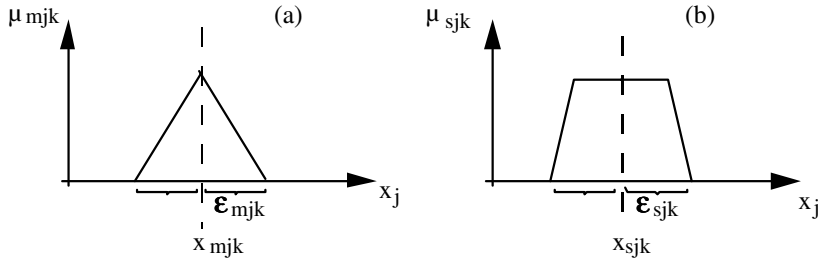


Figure 1. Presentation of membership functions.
(a) for measurement of state variable x_j at t_k (x_{mjk}) with estimated uncertainty ε_{mjk}
(b) for model simulation of state variable x_j at t_k (x_{sjk}) with acceptable inaccuracy ε_{sjk}

2.3. Global relationship

At last, the accomplishment degree r_i of the "static" experiment i through the dynamic experiment reads

$$r_i = \sup_{(k)} (\inf_{(Lij \in Ei)} (\sup_{(xj)} \min (\min (\mu_{mjk}(x_j), \mu_{sjk}(x_j)) , \mu_{Lij}(x_j)))).$$

3. Characteristics of the system

3.1. Initial experiments

Our purpose is to find the best composition of a yeast culture medium to obtain the higher lipid accumulation synthetised by yeasts.

Table 1. Characteristics of the 9 already realized experiments.

	Medium components (g/l)					**Factors**		
N°	Glucose	Glycerol	Stearin	Ammonium sulfate	Yeast extract	x	y	z
1	0	10.5	10.0	0.5	2.0	0.64	0.34	0.17
2	33.0	0	0	0.1	0.5	0.00	0.00	0.81
3	0	18.0	0	0.1	0.5	0.00	0.99	0.43
4	16.0	9.0	0	0.1	0.5	0.00	0.35	0.61
5	0	22.7	11.7	0.5	0.5	0.50	0.50	0.44
6	0	34.5	11.5	0.5	0.5	0.39	0.60	0.55
7	0	0	14.0	0.7	2.0	0.98	0.00	0.13
8	30.5	0	0	0.1	2.0	0.00	0.00	0.27
9	0	30.5	0	0.1	2.0	0.00	0.98	0.26

This synthesis is made when carbon substrates are present. Besides, nitrogen supply is known to be important for this kind of production; lipid accumulation by the yeast would begin when nitrogen exhaustion. Then, the synthesis begins when the [C]/[N] ratio is high (Gill et al., 1977).

At the beginning of the study, preliminary runs have been realized so as to test the ability of the chosen yeast, Yarrowia lipolytica, to accumulate lipids and its ability to grow on designated carbon substrates. Three of them have been chosen for their low costs and their interesting potentialities: glucose, raw glycerol (unpurified) and stearin (free fatty acids from animal fat). As a consequence, nine experiments, already done with the three chosen substrates, before the beginning of the experimental design, can be used supplying data. These ones and their characteristics are represented in table 1.

3.2. Factors

The study of the system is needed to define what kind of response is obtained for what kind of operating conditions. Factors are elements which can be modified by the user and constitute the entry variables of the experimental design. Three factors have been chosen, hence, and allowed to describe the culture medium that is carbon supply, type and quantity, and [C]/[N] value.

All three factors are calculated considering the total elementary concentration of carbon in the medium [C]. This quantity is obtained by adding carbon rate of each substrate. Considering the elementary mass composition, stearin, raw glycerol and glucose are respectively composed of 76%, 39% and 40% of carbon.

Then, [C] is:

$$[C] = 0.76[\text{stearin}] + 0.39[\text{glycerol}] + 0.40[\text{glucose}]$$

The first both factors, x and y, characterise the initial composition of carbon supply. x express the carbon rate brought by the stearin. The same is done for y which represents carbon supply of raw glycerol. x and y represent percentages and have values normed between 0.0 and 1.0.

$$x = \frac{0.76[\text{stearin}]}{[C]} \qquad y = \frac{0.39[\text{glycerol}]}{[C]}$$

Carbon supply due to glucose (40% of total glucose mass) is not represented as a factor but can be easily deducted from x and y:

$$[\text{glucose}] = \frac{[C]}{0.4}(1 - x - y)$$

The third factor, z, allows to know nitrogen supply in the medium. z represents the ratio between [C] and [N]. Tested values are from 0 to 340. This is the reason why a coefficient is added to norm it between 0 and 1.

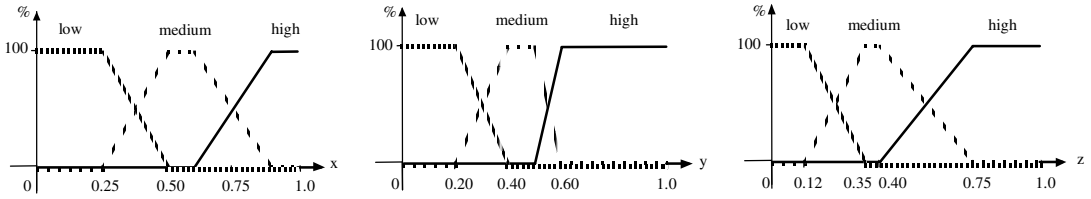
$$z = \frac{1}{340}\frac{[C]}{[N]}$$

Calculated values of factors are shown in table 1.

3.3. Membership functions and covering rate

To evaluate the covering rate of the domain, this one is cut in several fuzzy sets. By this way, each factor can belong with a known percentage to one of the third following levels: low, medium or high (Kuehm *et al.*, 1996).

These levels and the ensued percentages depend on the membership functions particular for each factor. These functions are defined separately for each factor by the user who choose them helped with his *a priori* knowledge. Thus, he can further a potentially interesting area by reducing the size of the function at this place. By this way, the more an area is narrow, the more experiments will have to be situated exactly in this area to obtain a satisfactory covering rate in there. On the contrary, a large area will generate a good rate with few experiments. The three functions are represented figure 2.



$$x = \frac{0.76[\text{stearin}]}{[C]} \qquad y = \frac{0.39[\text{glycerol}]}{[C]} \qquad z = \frac{[C]}{[N]}$$

Figure 2. Membership functions of factors.

For each experiment, the three factors are represented in a fuzzy way thanks to these membership functions. The covering rate is here calculated by the simplified relationship: $ri = \sup(k).\inf_{(Lij \in Ei)}.\mu_{Lij}(x_{mjk})$;

where the Ei are all the feasible combination of low, medium and high for the three factors.

The domain of study has been defined by the three factors which fixed the following restraints:

$$0.0 \le x, y, z \le 1.0$$

and $0.0 \le x+y \le 1.0$.

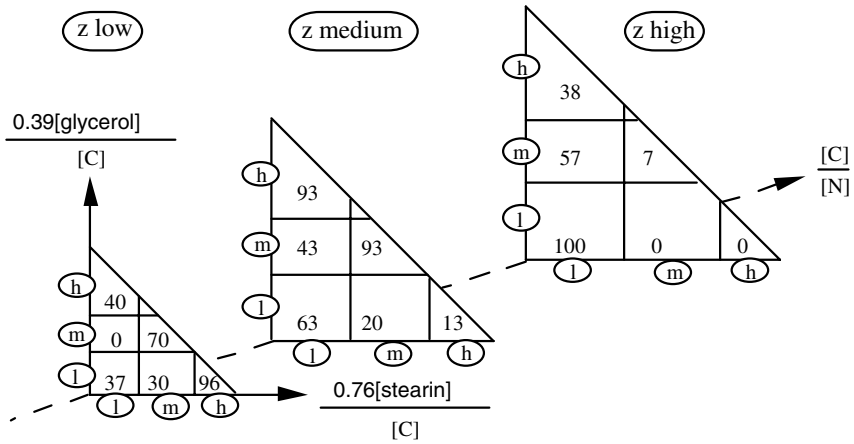


Figure 3. Representation of the domain with the covering rate of each area for 9 experiments.

Thus, the domain was represented as half a cube divided in 18 smaller fuzzy sets defined by levels low, medium and high of each of the three factors. The covering rate of the 18 areas has been determined thanks to fuzzy values of factors.

The figure 3 shows each area with its covering rate for the nine initial experiments.

3.4. Criteria

The aim is to optimize the production of single cell oil. Thus, it is necessary to improve the quantity and the quality of produced lipids as well as the efficiency of the culture. That is why, these three criteria have been chosen:

- ❑ productivity : $\mathbf{P} = \dfrac{[\text{Intracellular lipids}]}{[\text{Total biomass}] * \text{Time}}$
- ❑ yield : $\mathbf{Y} = \dfrac{[\text{Carbon in intracellular lipids}]}{[\text{Total consumed carbon}]}$
- ❑ composition : $\mathbf{Co} = \dfrac{[\text{Unsaturated Fatty acids}]}{[\text{Total Fatty acids}]}$

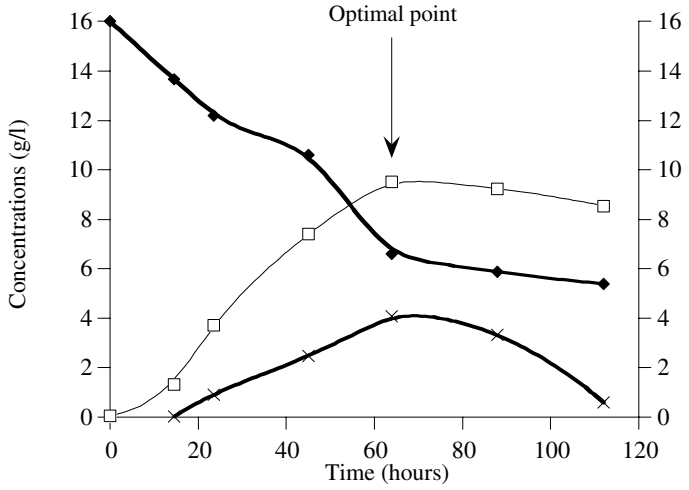


Figure 4. Example of choice of the optimal point for the calculation of criteria, ◆ stearin, □ biomass and × intracellular lipids.

These criteria are calculated for each experiment as described above. For the calculation, one optimal point has been chosen for each experiment when [intracellular lipids]/[biomass] was higher as shown in figure 4.

4. Supplementary experiments

4.1. Number of new experiments

Nine exploratory experiments have been realised to verify the feasibility of the study. Now, we have to add new runs to find the best possible design considering the nine first ones. The chosen model is a second degree polynomial model and need ten parameters. We had to add at least one experiment for the identification of

parameters. Four supplementary ones have been proposed to identify the error on the measure variance and to determine the confidence region of parameters.

4.2. Choice of new experiments

Helped with the F.D.E.D., five additional experiments allowed to obtain satisfactory covering rate. In this case, we have chosen not to use an optimization criteria but the human knowledge and decision support.

Covering rates obtained with the five new experiments added with the F.D.E.D. (see table 2) are represented in figure 5.

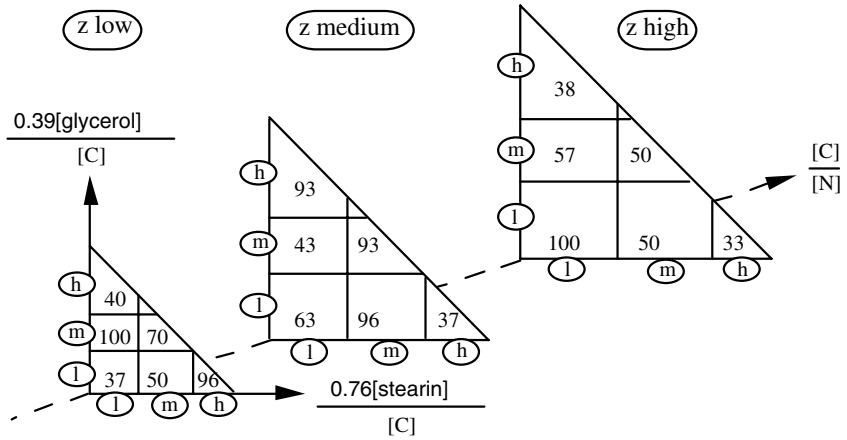


Figure 5. Representation of the domain with the covering rate of each area for 14 experiments.

4.3. Comparison with optimized ones

We decide to consider two statistical criteria to obtain new experiments. We choose the D-optimality criterion and the rotatability described by Khuri (1988). The addition of experiments considering these last two criteria is done with the help of a diploïd genetic algorithm (Perrin *et al.*, 1997). Table 2 presents the different complemented designs obtained.

Table 2. Comparison of four obtained designs.

	9 experiments + 5 F.D.E.D.			**9 experiments + 5 D-optimality**			**9 experiments + 5 rotatability**			**9 experiments + 3 D-optimality + 2 rotatability**		
Factors	x	y	z	x	y	z	x	y	z	x	y	z
10	0.71	0.29	0.43	0.00	1.00	1.00	0.66	0.34	1.00	0.00	1.00	1.00
11	0.70	0.30	0.93	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00
12	0.75	0.24	0.09	0.00	0.62	0.00	0.39	0.61	0.00	0.50	0.00	0.96
13	0.00	0.49	0.11	0.45	0.00	1.00	0.00	0.00	0.37	0.00	0.00	0.42
14	0.49	0.00	0.40	0.41	0.00	0.00	0.52	0.48	0.48	0.00	0.00	0.48
D-optimality	5.8e-4			8.8e-3			3.3e-5			2.3e-3		
Rotatability	16.2%			12.0%			37.0%			19.1%		

The comparison between the different results shows that the best compromise is obtained when addition of three D-optimality experiments and two ones to increase the rotatability. However, the calculation is then complex because of the necessity to use two different algorithms. To employ F.D.E.D. is only based on reflection. That is the reason why it will be applied in practice.

5. Modeling

5.1. Second degree polynomial model

Polynomials allow to reproduce, with any precision, any set of experimental values. The only restriction is to choose a high enough degree. In order to determine a polynomial model, it is in the nature of things to begin by a first degree model which is a linear model considering Hadamard. But, such a model shows quickly its limits because phenomena are not linear. For this reason, a second degree polynomial model has been chosen so as to be able to represent criteria in a satisfactory way. Therefore, for three factors, we needed to determine ten parameters. Indeed, the structure of the model is the following:

$$\mathbf{h = a_0 + a_1 x + a_2 y + a_3 z + a_4 x^2 + a_5 y^2 + a_6 z^2 + a_7 xy + a_8 xz + a_9 yz}$$

where **h** is the value of the criteria calculated for each experiment.
$\mathbf{a_0}$,..,$\mathbf{a_9}$ are parameters of the polynomial we have to determine.
x, **y** et **z** represent respectively the three factors (stearin, raw glycerol and [C]/[N] ratio).

At least, ten experiments were necessary for the calculation. The nine ones, defined in table 1, and the five ones, defined in table 2 with the F.D.E.D., were used.

5.2. Parameters calculation

The followed process is the same for the three criteria. **H** is the matrix of the criteria for the 14 experiments, **M** is the matrix of factors and **A** is the matrix of parameters which were unknown.

We write: $H = M.A + \varepsilon$ (1)
where ε represents the difference observed between measurement and prediction.

ε is made up of independent hazard N(0, v) where v is the unknown variance.

The estimation of the maximum likelihood is done on unknown parameters i.e. the matrix A and the variance v.

The equation (1) gives the way to estimate $\hat{A}$ and $\hat{v}$:

$$\hat{A} = (M^T.M)^{-1}.M^T.H$$
$$\hat{v} = (1/n_e).(H - M.\hat{A})^2$$ where n_e is the number of experiments.

In this case, $\hat{v}$ represents a biased estimation.

The confidence region of parameters is determined as described in the book written by Walter and Pronzato (1997). This method is useful for models which are non linear in their parameters. For these which are linear, this technique can be reduced to the one described by Draper and Smith (1981).

We can write:

$(A - \hat{A})^T.M^T.M.(A - \hat{A}) \leq (n_p/(n_e - n_p).F_\alpha(n_p, n_e - n_p).(H - M.\hat{A})^2$

where n_P is the number of parameters.

i.e.

$1/(n_p.v).(A - \hat{A})^T.M^T.M.(A - \hat{A})$ is a χ^2 with n_p liberty degrees

and

$1/((n_e - n_p).v).(H - M.\hat{A})^2$ is a χ^2 with $(n_e - n_p)$ liberty degrees.

We can deduce a calculation of the estimation without bias of v:

$$\hat{v}_{wb} = 1/(n_e - n_p).(H - M.\hat{A})^2.$$

The calculated values of $\hat{v}_{wb}$ are given in table 3 and those of parameters in table 4.

Table 3. Variance without bias for the three criteria, productivity (P), yield (Y) and composition (Co).

	Productivity	Yield	Composition
$\hat{v}_{wb}$	206.6	106.4	63.9

Table 4. Calculated parameters for the three criteria, productivity (P), yield (Y) and composition (Co).

	a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9
P	-0.95	124.6	27.9	57.3	-54.9	-49.6	-32.1	-158.2	-84.2	53.2
Y	4.5	62.4	-1.4	-6.1	-9.6	-16.0	4.5	-55.2	-55.6	56.1
Co	67.9	-151.3	-64.9	-11.2	90.5	64.3	16.0	51.0	22.7	-7.1

5.3. Model validity

Considering previous values of variance, the acceptable errors on the three criteria are:

± 28.2 for productivity;
± 20.2 for yield;
± 15.7 for composition.

Table 5 shows calculated results so as to verify the similarity of the model predictions and measurements.

Results show that no error exceeds the 95% confidence interval.

So as to verify the validity of the model, seven new experiments will be determined with the help of F.D.E.D.. These ones will be chosen to increase covering rates in any areas. They are presented in table 6. New covering rates are shown in figure 6.

Table 5. Comparison between values of the model and experimental ones for the fourteen experiments.

Exp	Factors			Productivity (**P**)			Yield (**Y**)			Composition (**Co**)		
N°.	**x**	**y**	**z**	meas.	calc.	**ε**	meas.	calc.	**ε**	meas.	calc.	**ε**
1	0.64	0.34	0.17	23.9	28.3	**-4.4**	27.6	22.5	**5.1**	8.0	5.2	**2.8**
2	0.0	0.0	0.81	26.6	24.4	**2.2**	3.6	2.6	**1.0**	70.0	69.4	**0.6**
3	0.0	0.99	0.43	11.2	19.7	**-8.5**	3.9	9.7	**-5.8**	70.0	61.8	**8.2**
4	0.0	0.35	0.61	33.2	37.1	**-3.9**	10.2	12.0	**-1.8**	49.5	50.7	**-1.2**
5	0.50	0.50	0.44	37.5	21.8	**15.7**	19.3	13.1	**6.2**	7.5	12.8	**-5.3**
6	0.39	0.60	0.55	30.0	22.4	**7.6**	18.7	12.4	**6.3**	14.6	20.0	**-5.4**
7	0.98	0.0	0.13	74.5	64.6	**9.9**	55.9	48.6	**7.3**	3.9	8.2	**-4.3**
8	0.0	0.0	0.27	10.6	12.0	**-1.4**	2.7	3.2	**-0.5**	65.5	66.0	**-0.5**
9	0.0	0.98	0.26	8.7	5.0	**3.7**	3.5	0.7	**2.8**	56.2	62.5	**-6.3**
10	0.71	0.29	0.43	16.5	30.9	**-14.4**	9.2	19.2	**-10.0**	14.9	7.3	**7.6**
11	0.70	0.30	0.93	14.3	15.6	**-1.3**	7.3	7.8	**-0.5**	19.7	19.6	**0.1**
12	0.75	0.24	0.09	28.9	37.4	**-8.5**	20.8	31.8	**-11.0**	5.2	3.1	**2.1**
13	0.0	0.49	0.11	13.1	9.5	**3.6**	3.8	2.3	**1.5**	51.4	50.1	**1.3**
14	0.49	0.0	0.40	47.8	48.2	**-0.4**	19.6	20.2	**-0.6**	18.2	18.0	**0.2**

Table 6. Comparison between values of the model and experimental ones for the seven new experiments.

Exp	Factors			Productivity (**P**)			Yield (**Y**)			Composition (**Co**)		
N°.	**x**	**y**	**z**	meas.	calc.	**ε**	meas.	calc.	**ε**	meas.	calc.	**ε**
15	0.00	0.00	0.38	7.9	16.2	**-8.3**	1.9	2.9	**-1.0**	70.0	66.0	**4.0**
16	0.00	1.00	0.37	9.3	13.4	**-4.1**	2.4	5.9	**-3.5**	62.0	62.8	**-0.8**
17	0.00	0.00	0.25	9.1	11.3	**-2.2**	4.3	3.3	**1.0**	73.0	66.1	**6.9**
18	0.97	0.00	0.11	69	65.2	**3.8**	44.1	49.5	**-5.4**	1.0	7.6	**-6.6**
19	0.00	0.97	0.18	9.6	-2.4	**12.0**	3.6	-3.3	**6.9**	62.0	62.8	**-0.8**
20	0.44	0.34	0.32	12.7	32.3	**-19.6**	11.5	16.3	**-4.8**	13.9	12.3	**1.6**
21	0.00	0.41	0.35	11.0	26.1	**-15.1**	6.0	7.8	**-1.8**	59.1	49.1	**10.0**

Again, errors do not exceed statistically admissible limits.

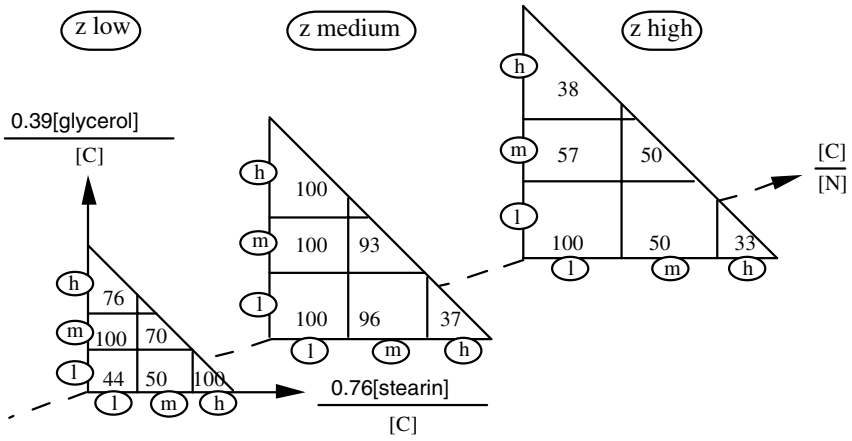


Figure 6. Representation of the domain with the covering rate of each area for 21 experiments.

6. Conclusion

The results show that F.D.E.D. proposed a good compromise between D-optimality and rotatability. However, if the design is supplemented, first, with three experiments which take into account D-optimality, and secondly, with two ones considering the rotatability, the efficiency of F.D.E.D. is a little bit lower. One of the principal advantage of F.D.E.D. is the simplicity to find rapidly supplementary experiments. The use of it needs few calculations and can be done without any optimization algorithm. Optimalities are decreased with F.D.E.D. but this technique is a good guide for heuristic design. Moreover, F.D.E.D. is well suited to determine exploratory experiments at the beginning of a new process and to find quickly generalization ones to validate a model.

7. References

Draper, N.R. and Smith, H. (1981). *Applied Regression Analysis*, John Wiley & Sons, Inc., 2nd edition.

Fonteix, C., Viennet, R. and Marc, I. (7-9 mai 1997). A new experimental design for multicriteria optimization: application to a biochemical synthesis. *2nd Int. Symposium on Mathematical modelling and simulation in agriculture and bio-industries*, IMACS, Budapest, 57-62.

Gill, C. Hall, M. and Ratledge, C. (1977). Lipid Accumulation in an Oleaginous Yeast with possession of ATP:Citrate lyase. *Applied and Environmental Microbiology*, 33, 231-239.

Khuri, A.I. (1988). A Measure of Rotatability for Response-Surface Designs. *Technometrics*, 30, 1, 95-104.

Kuehm, N., Fonteix, C., Marc, I. (1996). Fuzzy dynamic experimental sets for the determining of the validity of a state estimation. *RAIRO-APII-JESA Journal Européen des Systèmes Automatisés*, 30, 5, 737-753.

Perrin, E., Mandrille, A., Vivalda, J.C., Fonteix, C., Marc, I. (1997). Optimisation globale par stratégie d'évolution : technique utilisant la génétique des individus diploïdes. *RAIRO-Operation Research*, 31, 2, 151-201.

Walter, E. and Pronzato, L. (1997). Identification of parametric models from experimental data. *Communications and Control Engineering Series*, Springer, London.