Blocking and Confounding the 2^k Factorial Designs

Blocking and Confounding a 2^k Design

In any experiment, the variability arising from a **nuisance factor** can affect the results.

Sometimes a **nuisance factor** is **unknown** and **uncontrolled**:

we don't know that the factor exists and it may even be changing levels while we are conducting the experiment.

Experimental error

Randomization is the design technique used to guard against **nuisance factors**.

Nuisance factors probably affect the results but we are not interested in that effect.

Sometimes a **nuisance factor** is **known** and can be **controlled.**

Blocking is design technique used to eliminate the effect of a **nuisance factor**.

Blocking a Replicated 2^k Factorial Design

Evaluate the effect of the concentration of the su[...] concentration of the enzyme on the yield of an enzymatic reaction:

Substrate	Enzyme	x_S	x_E	Yield (	$n = 3$ replicates	
15 g/L	1 g/L	-1	-1	28	25	27
25 g/L	1 g/L	+1	-1	36	32	32
15 g/L	2 g/L	-1	+1	18	19	23
25 g/L	2 g/L	+1	+1	31	30	29

It was considered a **completely randomized experiment**

the order in which the 12 runs were made was random

or

the 12 runs were made at the same time

Blocking a Replicated 2^k Factorial Design

Evaluate the effect of the concentration of the substrate and concentration of the enzyme on the yield of an enzymatic reaction:

Substrate	Enzyme	x_S	x_E	Yield (%) $n = 3$ replicates		
15 g/L	1 g/L	-1	-1	28	25	27
25 g/L	1 g/L	+1	-1	36	32	32
15 g/L	2 g/L	-1	+1	18	19	23
25 g/L	2 g/L	+1	+1	31	30	29

Let's suppose it is not possible to run the 12 assays at the same time.

Running the 12 assays, one-at-a-time, in a random order, will take too long.

Blocking a Replicated 2^k Factorial Design

Evaluate the effect of the concentration of the substrate and concentration of the enzyme on the yield of an enzymatic reaction:

Substrate	Enzyme	x_S	x_E	Yield (%) $n = 3$ replicates		
15 g/L	1 g/L	[illegible]	-1	28	25	27
2[illegible]	[illegible]	[illegible]	-1	36	32	32
1[illegible]	[illegible]	[illegible]	+1	18	19	23
25[illegible]	[illegible]	[illegible]	+1	31	30	29

instead of running all assays homogeneously

Let's suppose it is not possible to run the 12 assays at the same time.

Running the 12 assays, one-at-a-time, in a random order, will take too long.

However, it is possible to run 4 assays at the same time.

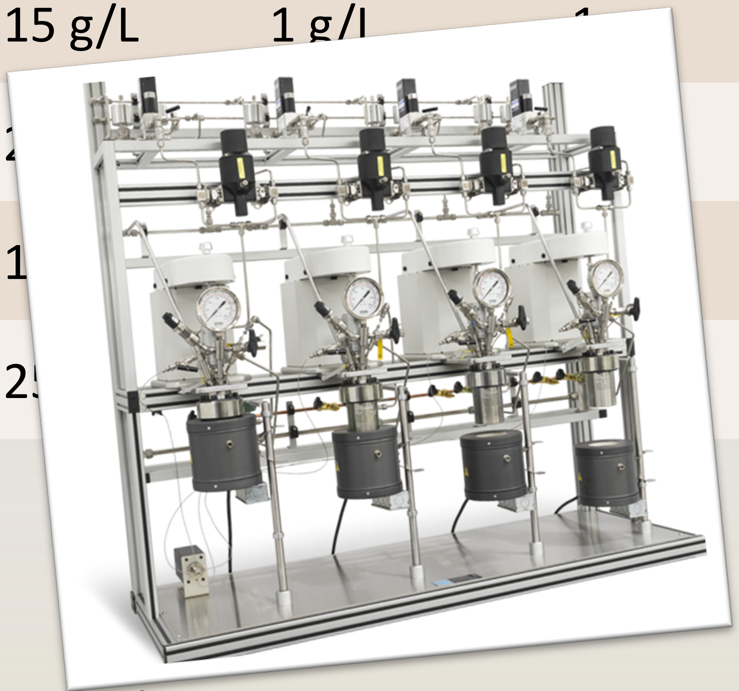
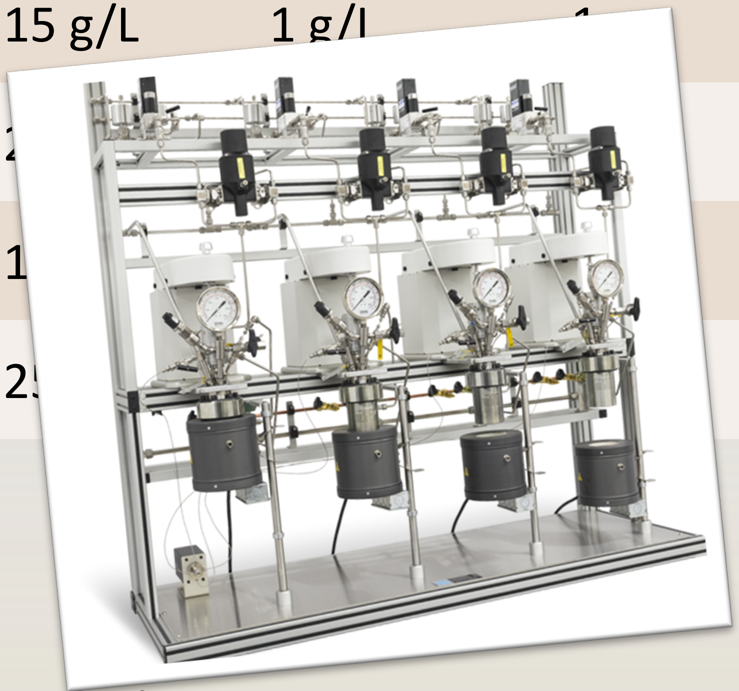


Image: https://www.parrinst.com/products/multi-reactor-systems/four-reactor-parallel-system/

Blocking a Replicated 2^k Factorial Design

Evaluate the effect of the concentration of the substrate ... me on the yield of an enzymatic reaction:

Substrate	Enzyme	x_S	x_E	Yield (%) $n = 3$ replicates		
15 g/L	1 g/L	-1	-1	28	25	27
2			-1	36	32	32
1			+1	18	19	23
25			+1	31	30	29
				B_1	B_2	B_3



3 blocks of 4 assays

Split the experiment, 12 assays, in 3 blocks of 4 assays.

A complete replicate will be run in each block.

...ys

Running the 12 assays, one-at-a-time, in a random order, will take too long.

However, it is possible to run 4 assays at the same time.

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Blocking a Replicated 2^k Factorial Design

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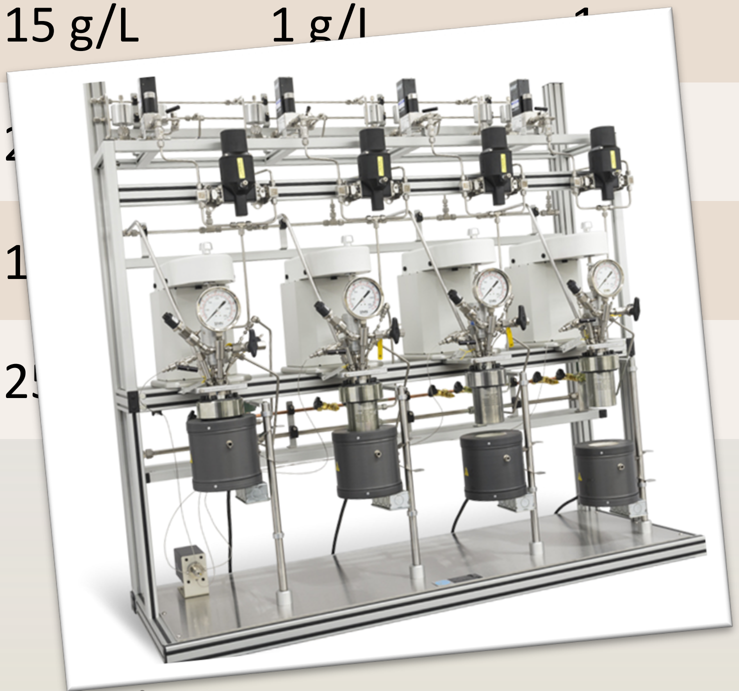
25[illegible]	[illegible]	[illegible]	+1	31	30	29
				B_1	B_2	B_3



3 blocks of 4 assays

Split the experiment, 12 assays, in 3 blocks of 4 assays.

A complete replicate will be run in each block.

Now the variability (noise) is not homogeneously distributed in the whole experiment, but is concentrated in the blocks.

Image: https://www.parrinst.com/products/multi-reactor-systems/four-reactor-parallel-system/

Blocking a Replicated 2^k Factorial Design

Evaluate the effect of the concentration of the substrate [illegible] me on the yield of an enzymatic reaction:

Substrate	Enzyme	x_S	x_E	Yield (%) $n = 3$ replicates		
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[illegible]	[illegible]	[illegible]	-1	36	32	32
[illegible]	[illegible]	[illegible]	+1	18	19	23
[illegible]	[illegible]	[illegible]	+1	31	30	29
				B_1	B_2	B_3

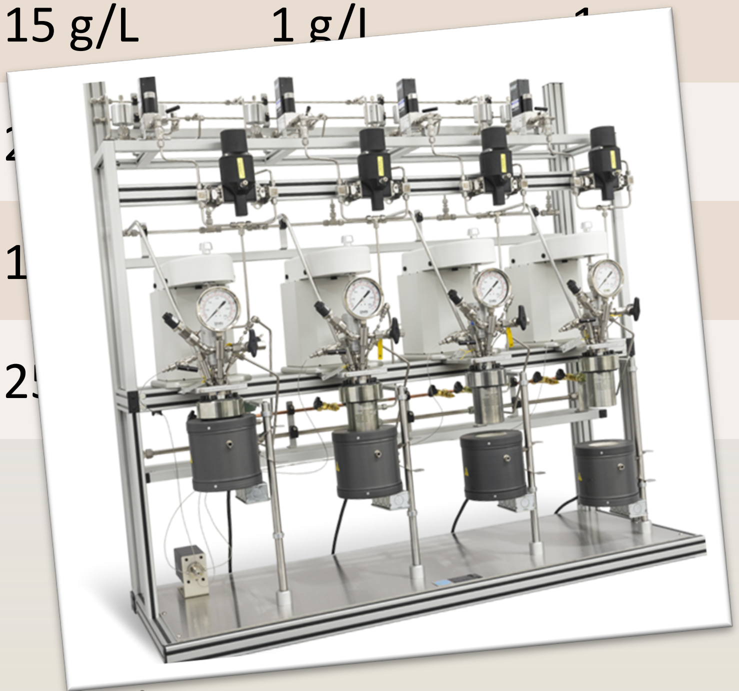

Image: https://www.parrinst.com/products/multi-reactor-systems/four-reactor-parallel-system/

Split the experiment, 12 assays, in 3 blocks of 4 assays.

A complete replicate will be run in each block.

Calculate the effect of the blocks for the Analysis of Variance

the replicates (blocks) are a 3rd factor in the experimental design

Blocking a Replicated 2^k Factorial Design: ANOVA

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F_0	p-value
Blocks	6.50	2	3.25	0.785	0.4978
Substrate	208.33	1	208.33	50.34	0.0004
Enzyme	75.00	1	75.00	18.12	0.0053
Interaction	8.33	1	8.33	2.013	0.2057
Error	24.84	6	4.14		
Total	323.00	11			

Blocking a Replicated 2^k Factorial Design: ANOVA

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F_0	p-value
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Enzyme	75.00	1	75.00	18.12	0.0053
Interaction	8.33	1	8.33	2.013	0.2057
Error	24.84	6	4.14		
Total	323.00	11			

nº of blocks−1

not signif

significant

Confounding in the 2^k Factorial Design

Sometimes it is **impossible** to run a complete replicate of a design in one block.

Confounding is used for splitting a complete factorial experiment in blocks.

Certain effects (usually high-order interactions) will be indistinguishable from, or **confounded** with, blocks.

Confounding the 2^k Factorial Design in Two Blocks

Single replicate of a 2^2 factorial design: 4 runs

Treat	A	B
(1)	-	-
a	+	-
b	-	+
ab	+	+

How can we divide 4 treatments in 2 blocks?

Limitation:
the equipment allows only two runs at a time.

each set of two runs = one block

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect			
Treat	I	A	B	AB
(1)	+	-	-	
a	+	+	-	
b	+	-	+	
ab	+	+	+	

treatments

Limitation:
the equipment allows only
two runs at a time.

each set of two runs = one block

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect			
Treat	I	A	B	AB
(1)	+	-	-	
a	+	+	-	
b	+	-	+	
ab	+	+	+	

identity column

Limitation:
the equipment allows only two runs at a time.

each set of two runs = one block

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect			
Treat	I	A	B	AB
(1)	+	-	-	+
a	+	+	-	-
b	+	-	+	-
ab	+	+	+	+

$AB = A \times B$

Factors
A and B

the signs + or −
are the ones of the coded variables

Limitation:
the equipment allows only
two runs at a time.

each set of two runs = one block

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect			
Treat	I	A	B	AB
(1)	+	-	-	+
a	+	+	-	-
b	+	-	+	-
ab	+	+	+	+

Table of Plus and Minus Signs is used to determine the Contrasts

$$C_A = ab + a - b - (1)$$

Limitation:
the equipment allows only two runs at a time.

each set of two runs = one block

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Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect			
Treat	I	A	B	AB
(1)	+	-	-	+
a	+	+	-	-
b	+	-	+	-
ab	+	+	+	+

Table of Plus and Minus Signs is used to determine the Contrasts

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

Limitation:
the equipment allows only two runs at a time.

each set of two runs = one block

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect			
Treat	I	A	B	AB
(1)	+	-	-	+
a	+	+	-	-
b	+	-	+	-
ab	+	+	+	+

Table of Plus and Minus Signs is used to determine the Contrasts

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

$$C_{AB} = ab + (1) - a - b$$

Limitation:
the equipment allows only two runs at a time.

each set of two runs = one block

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect				
Treat	I	A	B	AB	$Blocks$
(1)	+	-	-	+	1
a	+	+	-	-	1
b	+	-	+	-	2
ab	+	+	+	+	2

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

$$C_{AB} = ab + (1) - a - b$$

B_1

(1) a

B_2

b ab

$$C_{Block} = ab + b - a - (1)$$

Blocks are **confounded** with Factor B

We cannot distinguish the effect of the blocks from the effect of factor B.

Confounding the 2^k Factorial Design in Two Blocks

Table of P... Minus Signs for the 2^2 Design

	...rial Effect				
Treat	I	A		AB	$Blocks$
(1)	+	-	-	+	1
a	+	+			
b	+				
ab	+				

$C_A = ab + \ldots$

$C_B = ab + b - a - (1)$

$C_{AB} = \ldots + (1) - a - b$

$C_{Block} = a\ldots b - a - (1)$

Blocks are **confounded** with Factor B

We cannot distinguish the effect of the blocks from the effect of factor B.

Blocks must **NEVER** be **confounded** with **main effects**!!!!!

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect				
Treat	I	A	B	AB	*Blocks*
(1)	+	-	-	+	?
a	+	+	-	-	
b	+	-	+	-	
ab	+	+	+	+	

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

We can not confound the blocks with main effects

$$C_{AB} = ab + (1) - a - b$$

Confound the blocks with the interaction

Limitation:
the equipment allows only two runs at a time.

each set of two runs = one block

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Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect				
Treat	I	A	B	AB	$Blocks$
(1)	+	-	-	+	1
a	+	+	-	-	
b	+	-	+	-	
ab	+	+	+	+	1

B_1

(1) ab

treatments with the "+" sign in the AB column

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

$$C_{AB} = ab + (1) - a - b$$

W he
bl ts

$$C_{Blocks} = C_{AB}$$

Confound the blocks with the interaction

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect				
Treat	I	A	B	AB	$Blocks$
(1)	+	-	-	+	1
a	+	+	-	-	2
b	+	-	+	-	2
ab	+	+	+	+	1

B_1

(1) ab

B_2

a b

treatments with the "-" sign in the AB column

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

$$C_{AB} = ab + (1) - a - b$$

$$C_{Blocks} = C_{AB}$$

Confounding the 2^k Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^2 Design

	Factorial Effect				
Treat	I	A	B	AB	$Blocks$
(1)	+	-	-	+	1
a	+	+	-	-	2
b	+	-	+	-	2
ab	+	+	+	+	1

B_1

(1) ab

B_2

a b

$$C_A = ab + a - b - (1)$$

$$C_B = ab + b - a - (1)$$

$$C_{AB} = ab + (1) - a - b$$

$$C_{Blocks} = C_{AB}$$

$$C_{Blocks} = ab + (1) - a - b$$

blocks are confounded with interaction AB

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Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

Treatment	Factorial Effect							
	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

identity

Identity (I): all plus signs

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

Treatment	Factorial Effect								
	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

main effects

Identity (I): all plus signs

Main Effects (A, B, C):

high level $\rightarrow +$

low level $\rightarrow -$

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

Treatment	Factorial Effect								Block
	I	A	B	C	AB	AC	BC	ABC	
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

interaction effects

Identity (I): all plus signs

Main Effects (A, B, C):

high level $\rightarrow +$

low level $\rightarrow -$

Interaction Effects:

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

Identity (I): all plus signs

Main Effects (A, B, C):

high level $\rightarrow +$

low level $\rightarrow -$

Interaction Effects:

$$AB = A \times B$$

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	**Block**
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

Identity (I): all plus signs

Main Effects (A, B, C):

high level $\rightarrow +$

low level $\rightarrow -$

Interaction Effects:

$$AB = A \times B$$

$$AC = A \times C$$

$$BC = B \times C$$

$$ABC = A \times B \times C$$

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

Identity (I): all plus signs

Main Effects (A, B, C):

high level → +

low level → −

Interaction Effects:

$$AB = A \times B$$

$$AC = A \times C$$

$$BC = B \times C$$

$$ABC = A \times B \times C$$

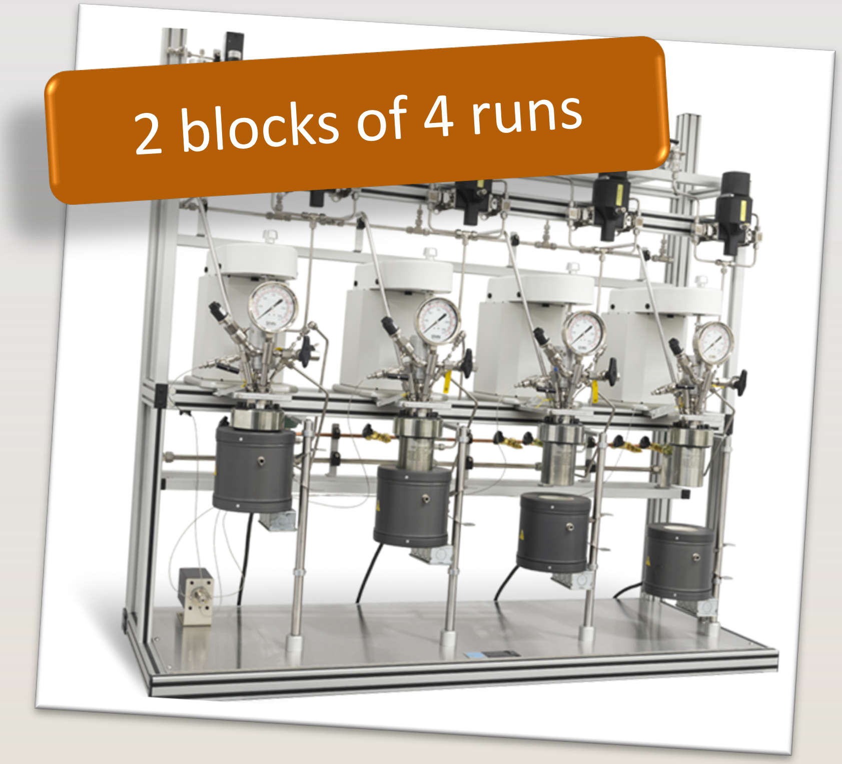
$$ABC = A \times BC = B \times AC = C \times AB$$

Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	-	-	+	+	+	-	
a	+	+	-	-	-	-	+	+	
b	+	-	+	-	-	+	-	+	
ab	+	+	+	-	+	-	-	-	
c	+	-	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	+	-	-	+	-	
abc	+	+	+	+	+	+	+	+	

We need to run a 2^3 design, 8 treatments, but the experimental apparatus allows only 4 runs at a time.



Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

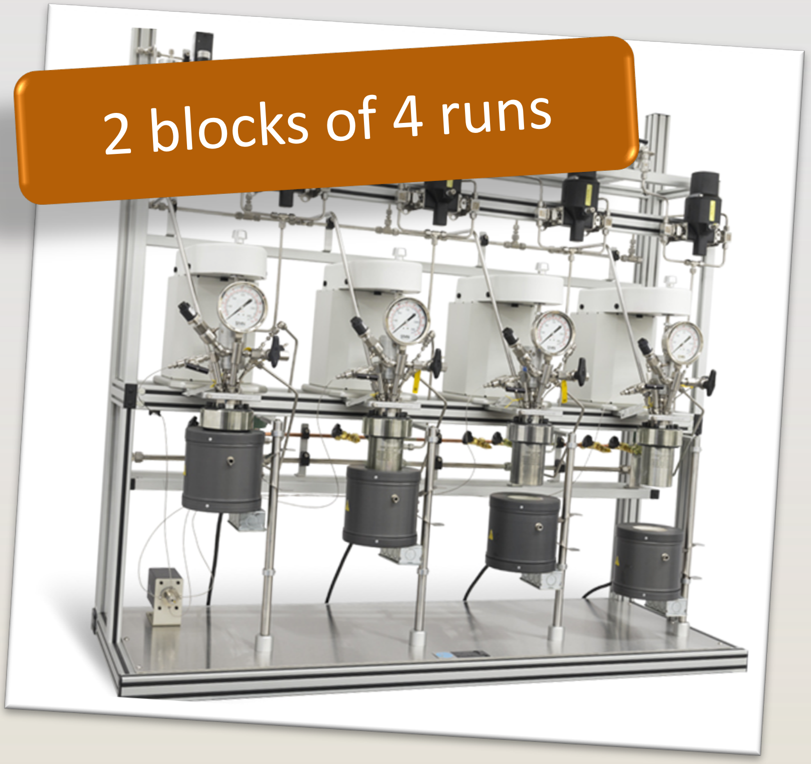
	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	Block
(1)	+	-	[illegible]	[illegible]	[illegible]	+	+	-	
a	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	+	+	
b	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	+	-	+	
ab	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	-	
c	[illegible]	[illegible]	-	+	+	-	-	+	
ac	+	+	-	+	-	+	-	-	
bc	+	-	+	[illegible]	[illegible]	[illegible]	[illegible]	-	
abc	+	+	+	+	+	+	+	+	

General Rule:
Always confound the highest-order interaction with the blocks.

$$C_{Blocks} = C_{ABC}$$

We need to run a 2^3 design, 8 treatments, but the experimental apparatus allows only 4 runs at a time.

2 blocks of 4 runs



Confounding the 2^3 Factorial Design in Two Blocks

Table of Plus and Minus Signs for the 2^3 Design

	Factorial Effect								
Treatment	I	A	B	C	AB	AC	BC	ABC	**Block**
(1)								-	2
a								+	1
b								+	1
ab								-	2
c						-	-	+	1
ac	+	+	-	+	-	+	-	-	2
bc	+	-	+					-	2
abc	+	+	+	+	+	+	+	+	1

$$
\begin{aligned}
C_{Blocks} &= B_1 - B_2 \\
&= a + b + c + abc \\
&\quad -(1) - ab - ac - bc \\
&= C_{ABC}
\end{aligned}
$$

$$C_{Blocks} = C_{ABC}$$

We need to run a 2^3 design, 8 treatments, but the experimental apparatus allows only 4 runs at a time.

2 blocks of 4 runs

Block 1 $(ABC+)$	Block 2 $(ABC-)$
a	(1)
b	ab
c	ac
abc	bc

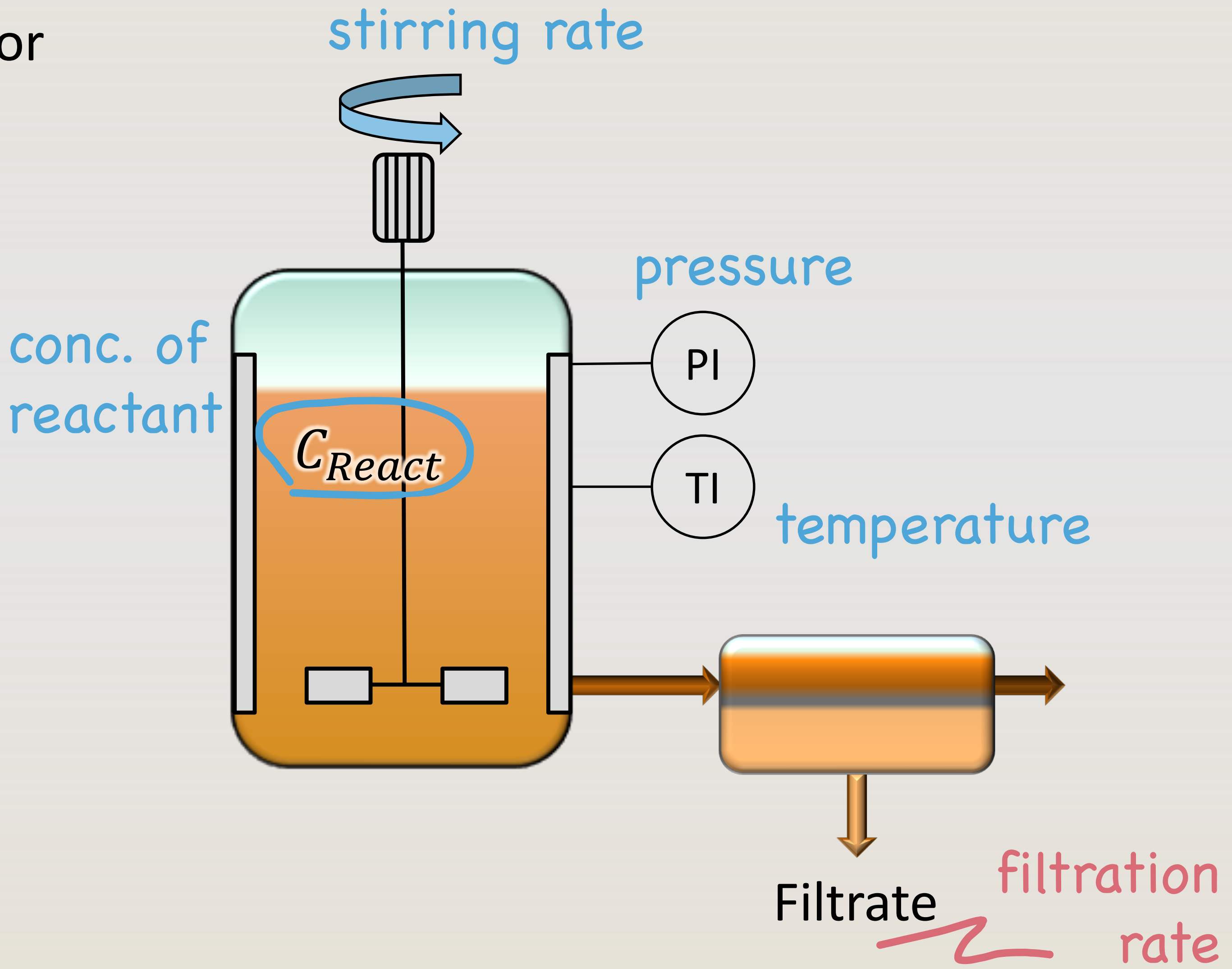
A Single Replicate of the 2^4 Design Split in Two Blocks

The drawing shows a chemical reactor followed by a filtration system.

The experimenter wants to evaluate how the variables:

- **Temperature**
- **Pressure**
- **Concentration of reactant**
- **Stirring rate**

affect filtration rate?



A Single Replicate of the 2^4 Design Split in Two Blocks

The drawing shows a chemical reactor followed by a filtration system.

The experimenter wants to evaluate how the variables:

- **Temperature**
- **Pressure**
- **Concentration of reactant**
- **Stirring rate**

affect filtration rate?

Table with factor levels:

Factor	**low level -1**	**high level +1**
A: Temperature (T, ˚C)	40	80
B: Pressure (P, atm)	1.5	2.5
C: Concentration (C, g/L)	50	100
D: Stirring rate (ω, rpm)	200	400

A Single Replicate of the 2^4 Design Split in Two Blocks

Treatment	A: T	B: P	C: C	D: ω	Filt. rate (L/min)
(1)	-	-	-	-	45
a	+	-	-	-	71
b	-	+	-	-	48
ab	+	+	-	-	65
c	-	-	+	-	68
ac	+	-	+	-	60
bc	-	+	+	-	80
abc	+	+	+	-	65
d	-	-	-	+	43
ad	+	-	-	+	100
bd	-	+	-	+	45
abd	+	+	-	+	104
cd	-	-	+	+	75
acd	+	-	+	+	86
bcd	-	+	+	+	70
$abcd$	+	+	+	+	96

2^4 design: 16 treatments

Let's suppose that one batch of raw material is not enough to run all the 16 runs

8 runs

8 runs

raw material

A Single Replicate of the 2^4 Design Split in Two Blocks

Treatment	A: T	B: P	C: C	D: ω	Filt. rate (L/min)
(1)	-	-	-	-	45
a	+	-	-	-	71
b	-	+	-	-	48
ab	+	+	-	-	65
c	-	-	+	-	68
ac	+	-	+	-	60
bc	-	+	+	-	80
abc	+	+	+	-	65
d	-	-	-	+	43
ad	+	-	-	+	100
bd	-	+	-	+	45
abd	+	+	-	+	104
cd	-	-	+	+	75
acd	+	-	+	+	86
bcd	-	+	+	+	70
$abcd$	+	+	+	+	96

2^4 design: 16 runs

Let's suppose that one batch of raw material is not enough

2^4 design confounded
2 blocks

8 runs

8 runs

raw material

A Single Replicate of the 2^4 Design Split in Two Blocks

Treatment	A: T	B: P	C: C	D: ω	ABCD	Filt. rate (L/min)
(1)	-	-	-	-	+	[illegible]
a	+	-	-	-	-	71
b	-	+	-	-	-	48
ab	+	+	-	-	+	65
c	-	-	+	-	-	68
ac	+	-	+	-	+	60
bc	-	+	+	-	+	80
abc	+	+	+	-	-	65
d	-	-	-	+	-	43
ad	+	-	-	+	+	100
bd	-	+	-	+	+	45
abd	+	+	-	+	-	104
cd	-	-	+	+	+	75
acd	+	-	+	+	-	86
bcd	-	+	+	+	-	70
$abcd$	+	+	+	+	+	96

$$ABCD = A \times B \times C \times D$$

des

General Rule:
Always confound the highest-order interaction with the blocks.

$$C_{Blocks} = C_{ABCD}$$

8 runs

8 runs

raw material

A Single Replicate of the 2^4 Design Split in Two Blocks

Treatment	A: T	B: P	C: C	D: ω	ABCD	Filt. rate (L/min)	Block
(1)	-	-	-	[illegible]	+	45	1
a	[illegible]	[illegible]	[illegible]	[illegible]	-	71	2
b	[illegible]	[illegible]	[illegible]	-	-	48	2
ab	+	+	-	-	+	65	1
c	-	-	+	-	-	68	2
ac	+	-	+	-	+	60	1
bc	-	+	+	-	+	80	1
abc	+	+	+	-	-	65	2
d	-	-	-	+	-	43	2
ad	+	-	-	+	+	100	1
bd	-	+	-	+	+	45	1
abd	+	+	-	+	-	104	2
cd	-	-	+	+	+	75	1
acd	+	-	+	+	-	86	2
bcd	-	+	+	+	-	70	2
$abcd$	+	+	+	+	+	96	1

$$C_{Blocks} = C_{ABCD}$$

Block 1
$(ABCD+)$
(1)
ab
ac
bc
ad
bd
cd
$abcd$

A

Block 2
$(ABCD-)$
a
b
c
abc
d
abd
acd
bcd

B

A Single Replicate of the 2^4 Design Split in Two Blocks

Treatment	A: T	B: P	C: C	D: ω	Block	Filt. rate
(1)	-	-	-	[illegible]	1	45
a	[illegible]	[illegible]	[illegible]	[illegible]	2	71
b	[illegible]	[illegible]	[illegible]	-	2	48
ab	+	+	-	-	1	65
c	-	-	+	-	2	68
ac	+	-	+	-	1	60
bc	-	+	+	-	1	80
abc	+	+	+	-	2	65
d	-	-	-	+	2	43
ad	+	-	-	+	1	100
bd	-	+	-	+	1	45
abd	+	+	-	+	2	104
cd	-	-	+	+	1	75
acd	+	-	+	+	2	86
bcd	-	+	+	+	2	70
$abcd$	+	+	+	+	1	96

$$C_{Blocks} = C_{ABCB}$$

completely randomised experiment

these results are homogeneous

Block 1 $(ABCD+)$	Block 2 $(ABCD-)$
(1)	a
ab	b
ac	c
bc	abc
ad	d
bd	abd
cd	acd
$abcd$	bcd

A Single Replicate of the 2^4 Design Split in Two Blocks

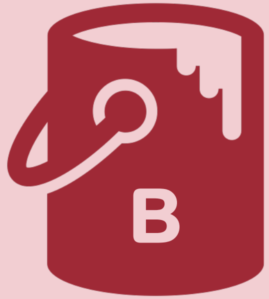
Treatment	A: T	B: P	C: C	D: ω	Block	Filt. rate
(1)	-	-	-	[illegible]	1	~~45~~ 25
a	[illegible]	[illegible]	[illegible]	[illegible]	2	71
b	[illegible]	[illegible]	[illegible]	-	2	48
ab	+	+	-	-	1	~~65~~ 45
c	-	-	+	-	2	68
ac	+	-	+	-	1	~~60~~ 40
bc	-	+	+	-	1	~~80~~ 60
abc	+	+	+	-	2	65
d	-	-	-	+	2	43
ad	+	-	-	+	1	~~100~~ 80
bd	-	+	-	+	1	~~45~~ 25
abd	+	+	-	+	2	104
cd	-	-	+	+	1	~~75~~ 55
acd	+	-	+	+	2	86
bcd	-	+	+	+	2	70
$abcd$	+	+	+	+	1	~~96~~ 76

$$C_{Blocks} = C_{ABCB}$$

Let's introduce an effect for the blocks

Batch A has low quality: results are 20 units lower

Block 1 $(ABCD+)$	Block 2 $(ABCD-)$
(1)	a
ab	b
ac	c
bc	abc
ad	d
bd	abd
cd	acd
$abcd$	bcd



A Single Replicate of the 2^4 Design Split in Two Blocks

Treatment	A: T	B: P	C: C	D: ω	Block	Filt. rate
(1)	-	-	-	[illegible]	1	25
a	[illegible]	[illegible]	[illegible]	[illegible]	2	71
b	[illegible]	[illegible]	[illegible]	-	2	48
ab	+	+	-	-	1	45
c	-	-	+	-	2	68
ac	+	-	+	-	1	40
bc	-	+	+	-	1	60
abc	+	+	+	-	2	65
d	-	-	-	+	2	43
ad	+	-	-	+	1	80
bd	-	+	-	+	1	25
abd	+	+	-	+	2	104
cd	-	-	+	+	1	55
acd	+	-	+	+	2	86
bcd	-	+	+	+	2	70
$abcd$	+	+	+	+	1	76

$$C_{Blocks} = C_{ABCB}$$

Batch A has low quality: results are 20 units lower

Block 1
$(ABCD+)$
(1)
ab
ac
bc
ad
bd
cd
$abcd$

Block 2
$(ABCD-)$
a
b
c
abc
d
abd
acd
bcd

The 2^4 Design Split in Two Blocks: Sum of Squares

```
> anova1 <- aov(FR1 ~ block + Factor_T*Factor_P*Factor_C*Factor_W, data = filtB)
> summary(anova1)
                            Df Sum Sq Mean Sq
block                        1 1387.6  1387.6
Factor_T                     1 1870.6  1870.6
Factor_P                     1   39.1    39.1
Factor_C                     1  390.1   390.1
Factor_W                     1  855.6   855.6
Factor_T:Factor_P            1    0.1     0.1
Factor_T:Factor_C            1 1314.1  1314.1
Factor_P:Factor_C            1   22.6    22.6
Factor_T:Factor_W            1 1105.6  1105.6
Factor_P:Factor_W            1    0.6     0.6
Factor_C:Factor_W            1    5.1     5.1
Factor_T:Factor_P:Factor_C   1   14.1    14.1
Factor_T:Factor_P:Factor_W   1   68.1    68.1
Factor_T:Factor_C:Factor_W   1   10.6    10.6
Factor_P:Factor_C:Factor_W   1   27.6    27.6
```

main factors

two-factor interactions

three-factor interactions

There is no interaction ABCD, as it is being used to estimate the effect of the blocks.

The 2^4 Design Split in Two Blocks: Sum of Squares

```
> anova1 <- aov(FR1 ~ block + Factor_T*Factor_P*Factor_C*Factor_W, data = filtB)
> summary(anova1)
                            Df Sum Sq Mean Sq
block                        1 1387.6  1387.6
Factor_T                     1 1870.6  1870.6
Factor_P                     1   39.1    39.1
Factor_C                     1  390.1   390.1
Factor_W                     1  855.6   855.6
Factor_T:F[illegible]        1    0.1     0.1
[illegible]                  1 1314.1  1314.1
[illegible]                  1   22.6    22.6
[illegible]                  1 1105.6  1105.6
[illegible]actor_W           1    0.6     0.6
Factor_C:Factor_W            1    5.1     5.1
Factor_T:Factor_P:Factor_C   1   14.1    14.1
Factor_T:Factor_P:Factor_W   1   68.1    68.1
Factor_T:Factor_C:Factor_W   1   10.6    10.6
Factor_P:Factor_C:Factor_W   1   27.6    27.6
```

some high sum of squares

the SS of the three-factor interactions are low

It is safe to use the three-factor interactions to evaluate the error

ANOVA Table: Main Factors and Two Factor Interactions

```
> anova2 <- aov(FR1 ~ block + (Factor_T+Factor_P+Factor_C+Factor_W)^2, data = filtB)
> summary(anova2)
                  Df Sum Sq Mean Sq F value  Pr(>F)   
block              1 1387.6  1387.6  46.156 0.00245 **
Factor_T           1 1870.6  1870.6  62.222 0.00140 **
Factor_P           1   39.1    39.1   1.299 0.31795   
Factor_C           1  390.1   390.1  12.975 0.02272 * 
Factor_W           1  855.6   855.6  28.459 0.00595 **
Factor_T:Factor_P  1    0.1     0.1   0.002 0.96582   
Factor_T:Factor_C  1 1314.1  1314.1  43.711 0.00271 **
Factor_T:Factor_W  1 1105.6  1105.6  36.775 0.00373 **
Factor_P:Factor_C  1   22.6    22.6   0.751 0.43518   
Factor_P:Factor_W  1    0.6     0.6   0.019 0.89781   
Factor_C:Factor_W  1    5.1     5.1   0.168 0.70257   
Residuals          4  120.3    30.1                   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

the block effect is significant

significant effects

combination of the three-factor interactions

ANOVA Table: Main Factors and Two Factor Interactions

```
> anova2 <- aov(FR1 ~ block + (Factor_T+Factor_P+Factor_C+Factor_W)^2, data = filtB)
> summary(anova2)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
block	1	1387.6	1387.6	46.156	0.00245	**
			1870.6	62.222	0.00140	**
			39.1	1.299	0.31795	
			390.1	12.975	0.02272	*
			855.6	28.459	0.00595	**
			0.1	0.002	0.96582	
			1314.1	43.711	0.00271	**
			1105.6	36.775	0.00373	**
	1	22.6	22.6	0.751	0.43518	
Factor_P:Factor_W	1	0.6	0.6	0.019	0.89781	
Factor_C:Factor_W	1	5.1	5.1	0.168	0.70257	
Residuals	4	120.3	30.1			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

By performing backwards elimination of the non-significant effects, we can have a final ANOVA table only with the significant effects.

the block effect is significant

significant effects

combination of the three-factor interactions

ANOVA Table: Significant Effects

```
> anova3 <- aov(FR1 ~ block + Factor_T+Factor_C+Factor_W + Factor_T:Factor_C + Facto
> summary(anova3)
                  Df Sum Sq Mean Sq F value   Pr(>F)    
block              1 1387.6  1387.6   66.58 1.89e-05 ***
Factor_T           1 1870.6  1870.6   89.76 5.60e-06 ***
Factor_C           1  390.1   390.1   18.72 0.001915 ** 
Factor_W           1  855.6   855.6   41.05 0.000124 ***
Factor_T:Factor_C  1 1314.1  1314.1   63.05 2.35e-05 ***
Factor_T:Factor_W  1 1105.6  1105.6   53.05 4.65e-05 ***
Residuals          9  187.6    20.8                     
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
```

we have exactly the same significant effects of the design run in homogeneous conditions

Run the regression models e check the model adequacy

A Single Replicate of the 2^4 Design Split in Two Blocks: Regression Model

```
> coded.model <- lm(FR1 ~ xT + xC+ xW + xT*xC + xT*xW, data=filtB)
> summary(coded.model)

Call:
lm(formula = FR1 ~ xT + xC + xW + xT * xC + xT * xW, data = filtB)

Residuals:
     Min       1Q   Median       3Q      Max 
-14.3750  -9.5937  -0.3125   9.4687  13.8750 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   60.062      3.138  19.143 3.29e-09 ***
xT            10.812      3.138   3.446  0.00627 ** 
xC             4.938      3.138   1.574  0.14664    
xW             7.313      3.138   2.331  0.04201 *  
xT:xC         -9.062      3.138  -2.888  0.01615 *  
xT:xW          8.312      3.138   2.649  0.02434 *  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 12.55 on 10 degrees of freedom
Multiple R-squared:  0.7785,	Adjusted R-squared:  0.6677 
F-statistic: 7.029 on 5 and 10 DF,  p-value: 0.004608
```

A Single Replicate of the 2^4 Design Split in Two Blo

```
> coded.model <- lm(FR1 ~ xT + xC+ xW + xT*xC + xT*xW,
> summary(coded.model)

Call:
lm(formula = FR1 ~ xT + xC + xW + xT * xC + xT * xW, d

Residuals:
     Min       1Q   Median       3Q      Max
-14.3750  -9.5937  -0.3125   9.4687  13.8750

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   60.062      3.138  19.143 3.29e-09 ***
xT            10.812      3.138   3.446  0.00627 **
xC             4.938      3.138   1.574  0.14664
xW             7.313      3.138   2.331  0.04201 *
xT:xC         -9.062      3.138  -2.888  0.01615 *
xT:xW          8.312      3.138   2.649  0.02434 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 12.55 on 10 degrees of freedom
Multiple R-squared:  0.7785,    Adjusted R-squared:  0.6677
F-statistic: 7.029 on 5 and 10 DF,  p-value: 0.004608
```

the regression coefficients are the same from the example using homogeneous conditions

factors are showing exactly the same effects

Unlike fixed factors as temperature, pH, concentration, type of enzyme, the effect of the blocks is not constant and cannot be predicted by a model.

the regression model does not take into account the blocks

The R^2 is too low to build contour plots. The model explains only 77.8% of the data variability.

lower than the one from the homogeneous conditions ($R^2 = 0.966$)

A Single Replicate of the 2^4 Design Split in Two Blo

```
> coded.model1 <- lm(FR1 ~ xT + xC+ xW + xT*xC + xT*xW, data=filtB)
.model)

~ xT + xC + xW + xT * xC + xT * xW, data = filtB)

           Q    Median       3Q      Max
     -9.5937   -0.3125   9.4687  13.8750

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   60.062      3.138  19.143 3.29e-09 ***
xT            10.812      3.138   3.446  0.00627 **
xC             4.938      3.138   1.574  0.14664
xW             7.313      3.138   2.331  0.04201 *
xT:xC         -9.062      3.138  -2.888  0.01615 *
xT:xW          8.312      3.138   2.649  0.02434 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 12.55 on 10 degrees of freedom
Multiple R-squared:  0.7785,    Adjusted R-squared:  0.6677
F-statistic: 7.029 on 5 and 10 DF,  p-value: 0.004608
```

Which type of graphs can be used to illustrate the results?

factors are showing exactly the same effects

Unlike fixed factors as temperature, pH, concentration, type of enzyme, the effect of the blocks is not constant and cannot be predicted by a model.

the regression model does not take into account the blocks

The R^2 is too low to build contour plots. The model explains only 77.8% of the data variability.

lower than the one from the homogeneous conditions ($R^2 = 0.966$)

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A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

```r
# bar plots
library(ggplot2)
```

package for data visualization in R

```r
# Temperature x Stirring Rate
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=W)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  coord_cartesian(ylim = c(0, 100)) +
  theme_bw() +
  facet_grid(.~W, labeller = label_parsed) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)

# Temperature x Concentration
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=C)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  coord_cartesian(ylim = c(0, 100)) +
  theme_bw() +
  facet_grid(.~C, labeller = label_parsed) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)
```

A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

bar graph with the mean

data file

x axis

y axis

the bars will be colored according the stirring rate (ω)

error bar for the standard error of the means

y limit

x and y lables

splitting the graphs by the stirring rate: one graph for each ω

```
# bar plots
library(g

# Temperature x Stirring Rate
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=W)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  coord_cartesian(ylim = c(0, 100)) +
  theme_bw() +
  facet_grid(.~W, labeller = label_parsed) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)

# Temperature x Concentration
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=C)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  coord_cartesian(ylim = c(0, 100)) +
  theme_bw() +
  facet_grid(.~C, labeller = label_parsed) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)
```

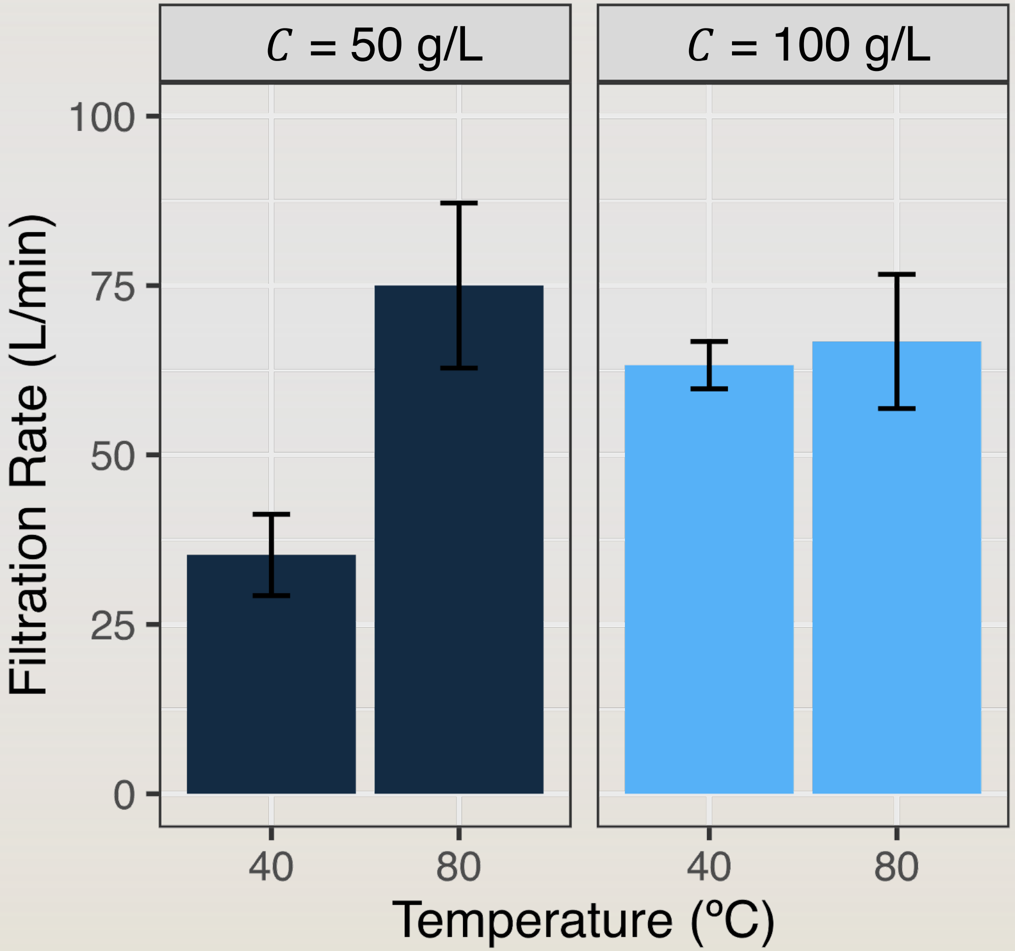
A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

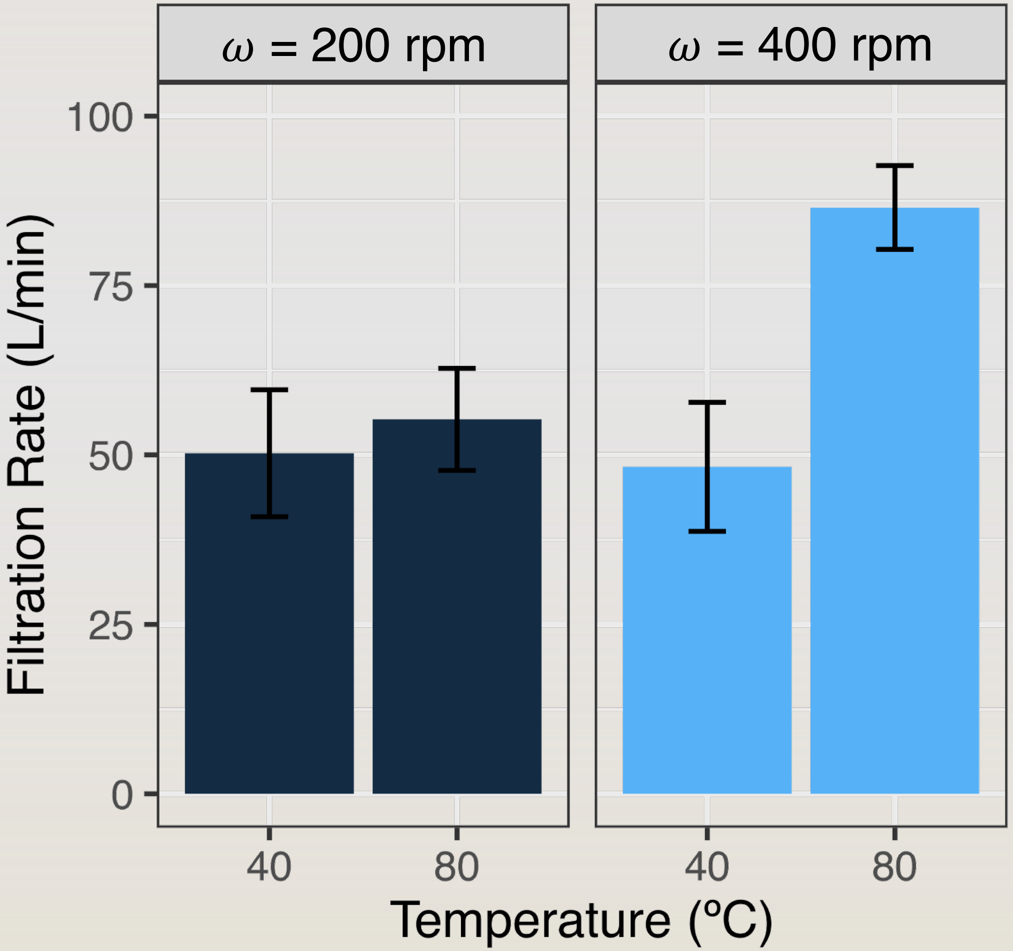
```
# bar plots
library(ggplot2)

# Temperature x Stirring Rate
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=W)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  coord_cartesian(ylim = c(0, 100)) +
  theme_bw() +
  facet_grid(.~W, labeller = label_parsed) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)

# Temperature x Concentration
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=C)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  coord_cartesian(ylim = c(0, 100)) +
  theme_bw() +
  facet_grid(.~C, labeller = label_parsed) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)
```

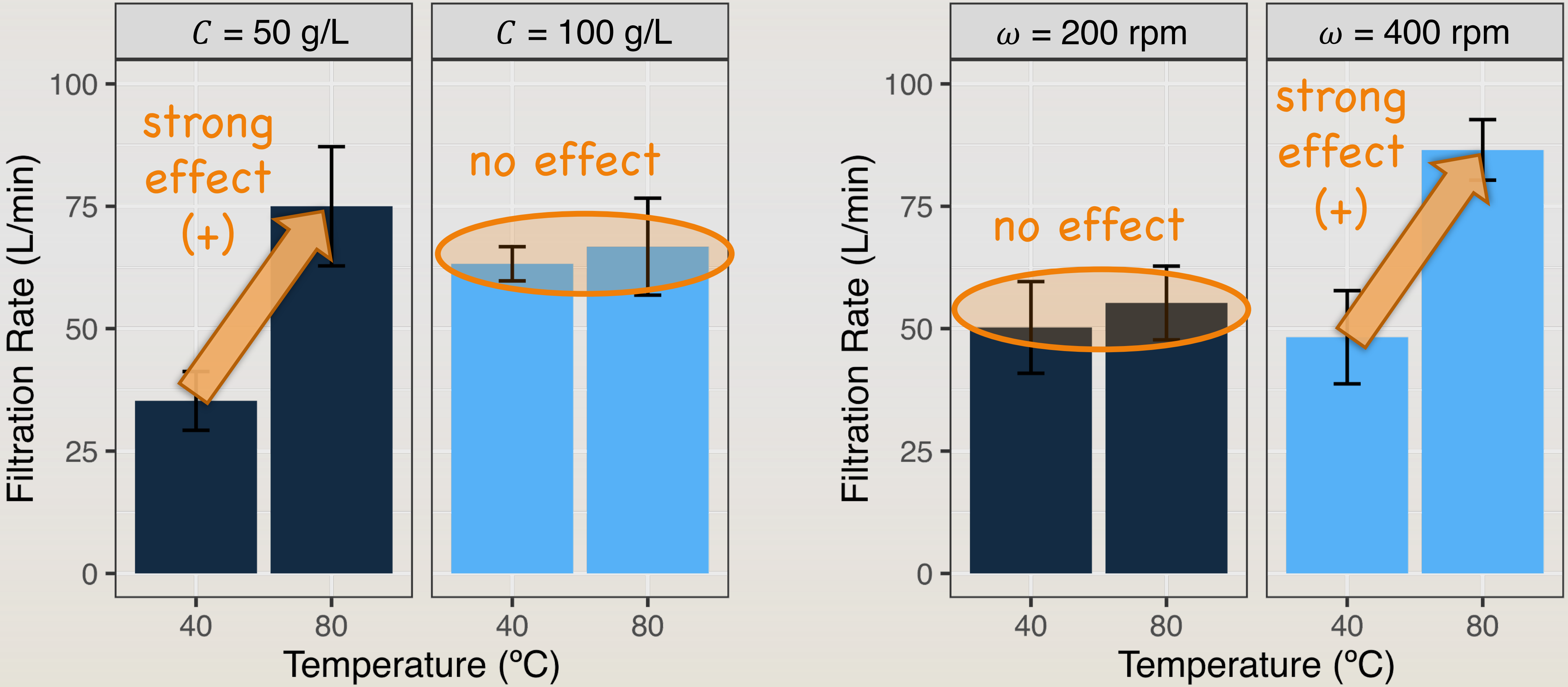
A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs





A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

Effect of Temperature:



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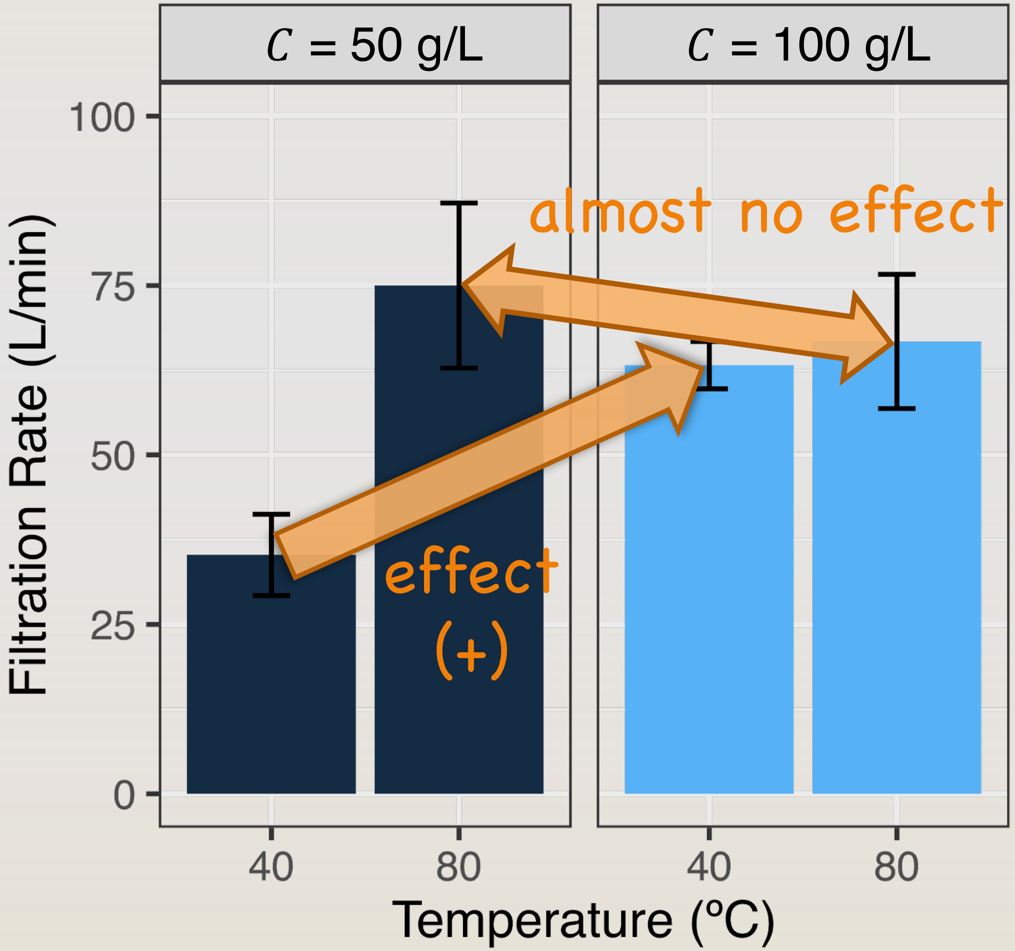
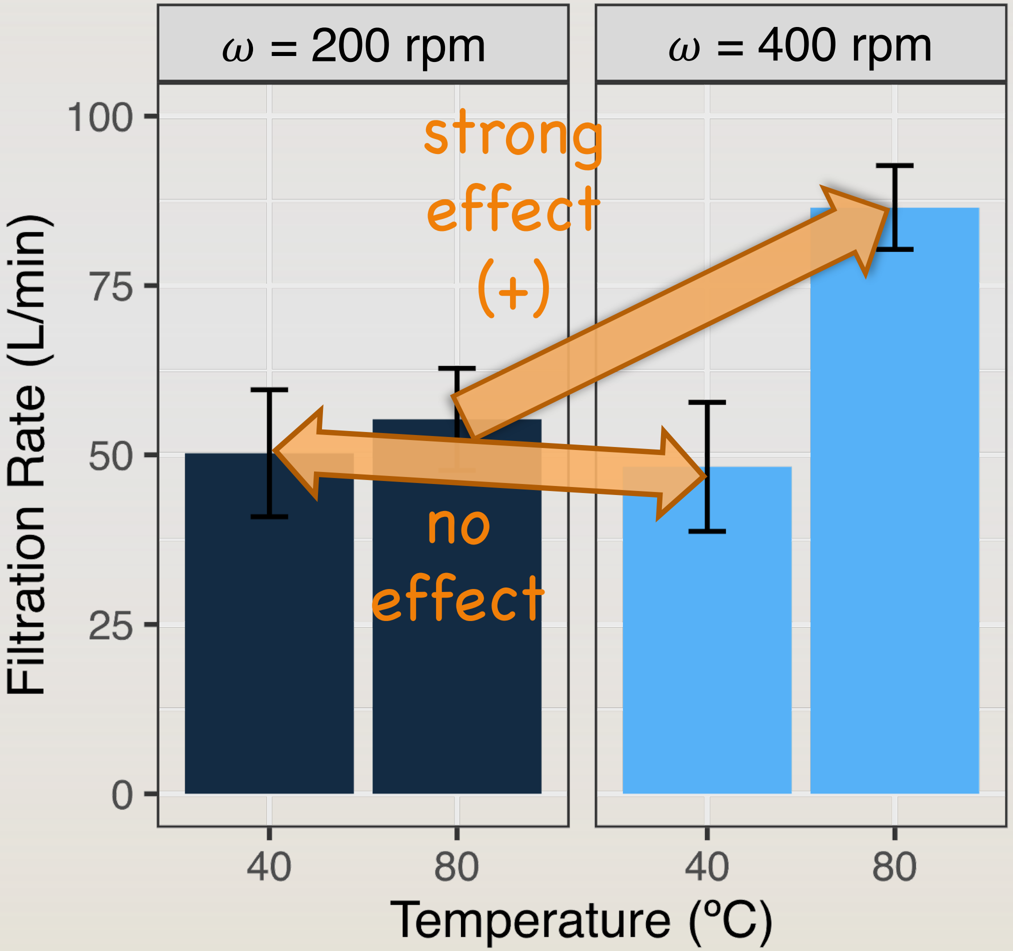
A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

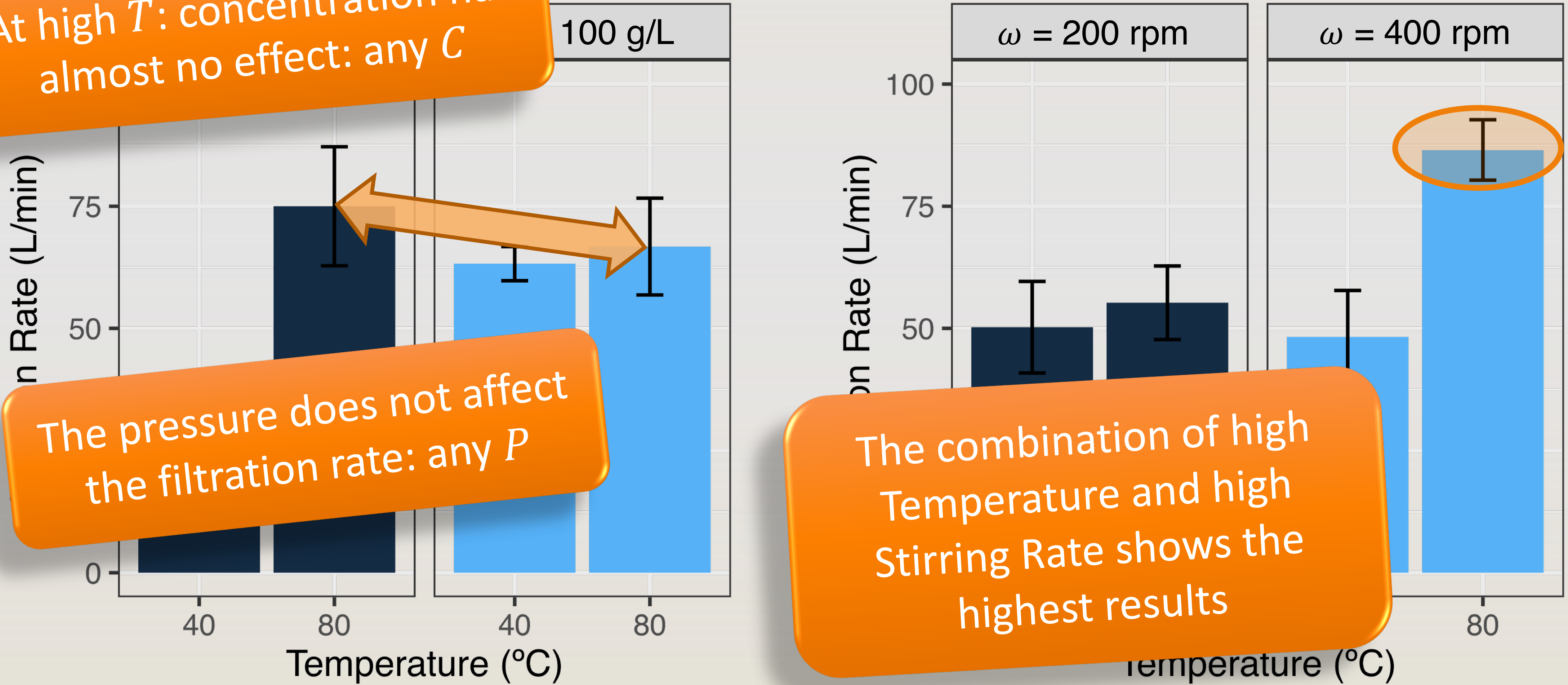
Effect of Concentration:



Effect of Stirring Rate:



A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs



At high T: concentration has almost no effect: any C

The pressure does not affect the filtration rate: any P

The combination of high Temperature and high Stirring Rate shows the highest results

A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

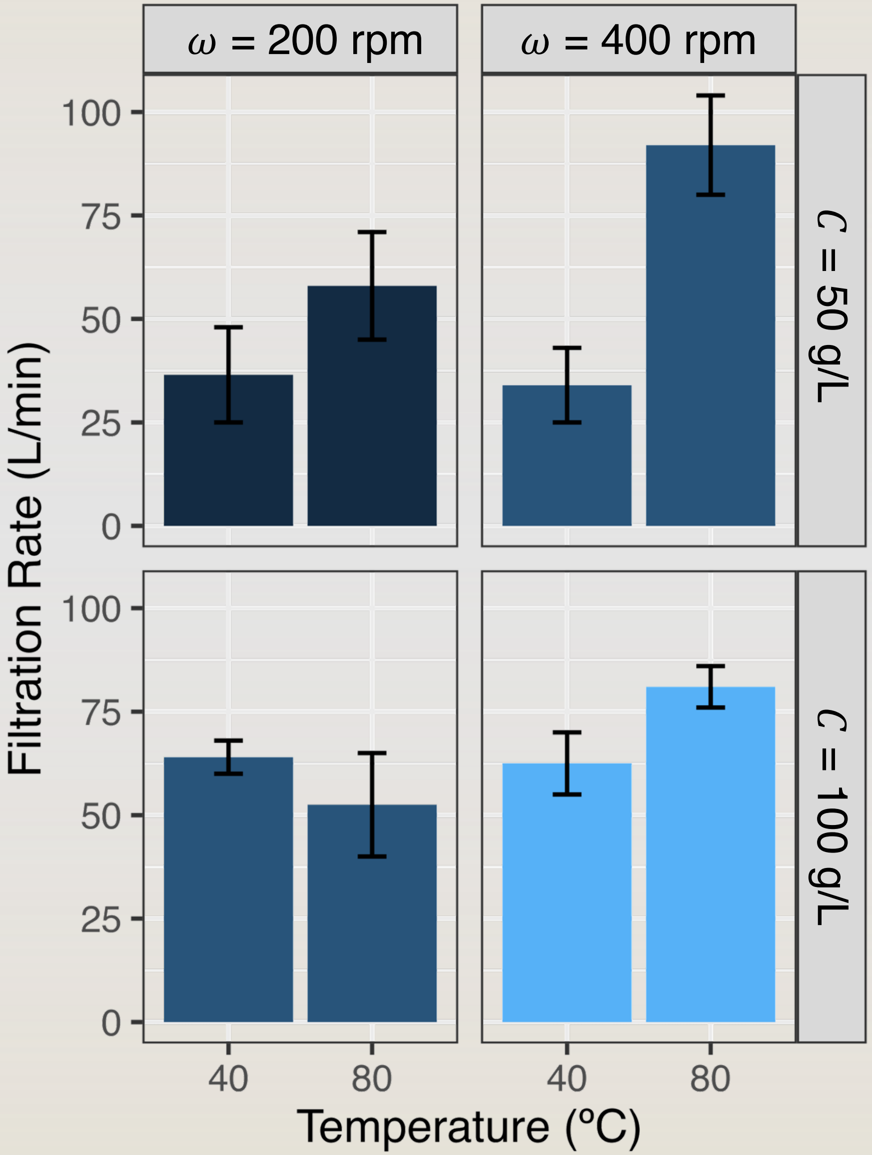
Another way of presenting the experimental data is splitting the results by temperature, stirring rate and concentration at the same time:

```
# Temperature x Concentration x Stirring rate
p <- ggplot(filtB, aes(x=Factor_T,y=FR1, fill=W*C)) +
  stat_summary(fun.y = mean, geom = "bar") +
  stat_summary(fun.data = mean_se , geom = "errorbar", width=0.2) +
  theme_bw() +
  facet_grid(C~.~W, labeller = label_both) +
  xlab("Temperature (°C)") + ylab("Filtration Rate (L/min)")
print(p)
```

splitting the plots by the concentration and stirring rate: one plot for each $C \times \omega$ combination

A Single Replicate of the 2^4 Design Split in Two Blocks: Bar Graphs

Another way of presenting the experimental data is splitting the results by temperature, stirring rate and concentration at the same time:



Example: 2^4 Factorial Design with Non-homogeneous Data

Treatment	A	B	C	D	r. mat	Filt. rate (L/min)
(1)	-	-	-	-	B	45
a	+	-	-	-	A	71
b	-	+	-	-	A	48
ab	+	+	-	-	A	65
c	-	-	+	-	B	68
ac	+	-	+	-	A	60
bc	-	+	+	-	A	80
abc	+	+	+	-	B	65
d	-	-	-	+	B	43
ad	+	-	-	+	A	100
bd	-	+	-	+	B	45
abd	+	+	-	+	B	104
cd	-	-	+	+	B	75
acd	+	-	+	+	A	86
bcd	-	+	+	+	B	70
$abcd$	+	+	+	+	A	96

random

Why blocking is important?

two batches of raw material:

Let's suppose we have skipped the class about blocks and decided to distribute the raw material randomly among the runs.

Example: 2^4 Factorial Design with Non-homogeneous Data

Treatment	A	B	C	D	r. mat	Filt. rate (L/min)
(1)	-	-	-	-	B	45
a	+	-	-	-	A	~~71~~ 51
b	-	+	-	-	A	~~48~~ 28
ab	+	+	-	-	A	~~65~~ 45
c	-	-	+	-	B	68
ac	+	-	+	-	A	~~60~~ 40
bc	-	+	+	-	A	~~80~~ 60
abc	+	+	+	-	B	65
d	-	-	-	+	B	43
ad	+	-	-	+	A	~~100~~ 80
bd	-	+	-	+	B	45
abd	+	+	-	+	B	104
cd	-	-	+	+	B	75
acd	+	-	+	+	A	~~86~~ 66
bcd	-	+	+	+	B	70
$abcd$	+	+	+	+	A	~~96~~ 76

random

Why blocking is important?

two batches of raw material:

low quality: results are 20 units lower

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Example: 2^4 Factorial Design with Non-homogeneous Data

Treatment	A	B	C	D	r. mat	Filt. rate (L/min)
(1)	-	-	-	-	B	45
a	+	-	-	-	A	51
b	-	+	-	-	A	28
ab	+	+	-	-	A	45
c	-	-	+	-	B	68
ac	+	-	+	-	A	40
bc	-	+	+	-	A	60
abc	+	+	+	-	B	65
d	-	-	-	+	B	43
ad	+	-	-	+	A	80
bd	-	+	-	+	B	45
abd	+	+	-	+	B	104
cd	-	-	+	+	B	75
acd	+	-	+	+	A	66
bcd	-	+	+	+	B	70
$abcd$	+	+	+	+	A	76

random

Why blocking is important?

two batches of raw material:

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Let's suppose we have skipped the class about blocks and decided to distribute the raw material randomly among the runs.

Example: 2^4 Factorial Design with Non-homogeneous Data

```
> anova5 <- aov(FR2 ~ Factor_T*Factor_P*Factor_C*Factor_W, data = filtB)
> summary(anova5)
                                  Df Sum Sq Mean Sq
Factor_T                           1  540.6   540.6
Factor_P                           1   39.1    39.1
Factor_C                           1  390.1   390.1
Factor_W                           1 1540.6  1540.6
Factor_T:Factor_P                  1  410.1   410.1
Factor_T:Factor_C                  1 1314.1  1314.1
Factor_P:Factor_C                  1   22.6    22.6
Factor_T:Factor_W                  1  540.6   540.6
Factor_P:Factor_W                  1   85.6    85.6
Factor_C:Factor_W                  1  150.1   150.1
Factor_T:Factor_P:Factor_C         1   14.1    14.1
Factor_T:Factor_P:Factor_W         1    3.1     3.1
Factor_T:Factor_C:Factor_W         1  175.6   175.6
Factor_P:Factor_C:Factor_W         1  232.6   232.6
Factor_T:Factor_P:Factor_C:Factor_W 1   52.6    52.6
```

Complete ANOVA

Three-factor interactions are rare

some three-factor interactions have substantially high sum of squares.

Example: 2^4 Factorial Design with Non-homogeneous Data

```
> anova9 <- aov(FR2 ~ Factor_T*Factor_C + Factor_W, data = f
> summary(anova9)
                  Df Sum Sq Mean Sq F value  Pr(>F)
Factor_T           1  540.6   540.6   3.446 0.09038 .
Factor_C           1  390.1   390.1   2.486 0.14314
Factor_W           1 1540.6  1540.6   9.820 0.00951 **
Factor_T:Factor_C  1 1314.1  1314.1   8.376 0.01460 *
Residuals         11 1725.7   156.9
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

ANOVA Table after step-by-step elimination

significant

We have missed most significant factors and interactions!

Example: 2^4 Factorial Design with Non-homogeneous Data

```
> anova9 <- aov(FR2 ~ Factor_T*Factor_C + Factor_W, data = f
> summary(anova9)

Factor_T
Factor_C
Factor_W
Factor_T:Factor_C   1 1314.1   1314.1
Residuals          11 1725.7    156.9
---
```

When using blocks we are able to withdraw some known source of variability from the error.

ANOVA Table after step-by-step elimination

Mean Squares of the Residuals: MS_E

7.5 times higher

```
> anova3 <- aov(FR1 ~ block + Factor_T+Factor_C+Factor_W
> summary(anova3)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
block	1	1387.6	1387.6	66.58	1.89e-05	***
Factor_T	1	1870.6	1870.6	89.76	5.60e-06	***
Factor_C	1	390.1	390.1	18.72	0.001915	**
Factor_W	1	855.6	855.6	41.05	0.000124	***
Factor_T:Factor_C	1	1314.1	1314.1	63.05	2.35e-05	***
Factor_T:Factor_W	1	1105.6	1105.6	53.05	4.65e-05	***
Residuals	9	187.6	20.8			

Some Important Remarks:

The problem of raw material:

We could have mixed them before the runs and avoid the non-homogeneity.

If we can not avoid some non-homogeneity, it has to be addressed using blocks:

Image by michelprado from Pixabay

Image by tigerlily713 from Pixabay

Image by ThisIsEngineering from Pexels

The problem with temperature:

Experimenters tend to group the runs at same temperature together, usually due to equipment limitation or simply because it is easier.

Some Important Remarks:

The problem of raw material:

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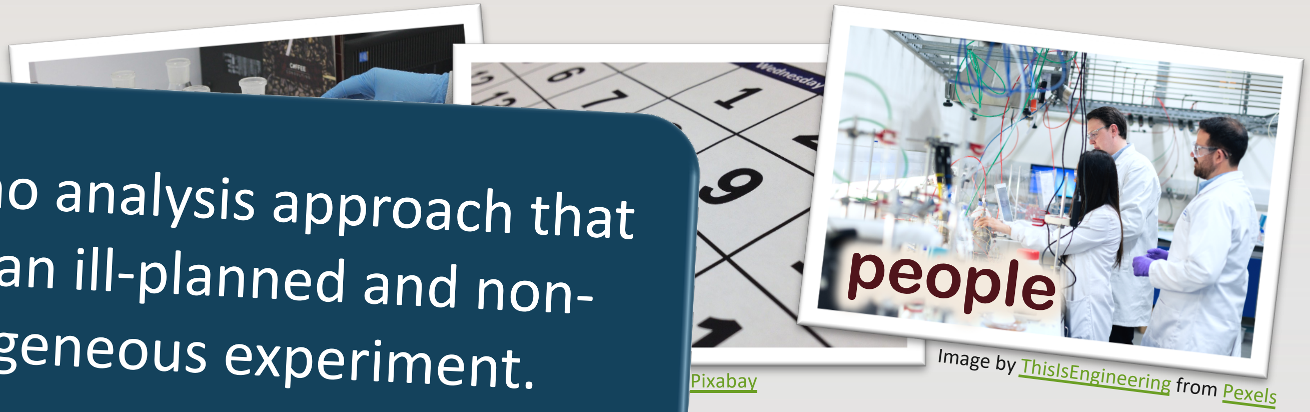

Pixabay

Image by ThisIsEngineering from Pexels

There is no analysis approach that "saves" an ill-planned and non-homogeneous experiment.

The problem with temperature:

Experimenters tend to group the runs at same temperature together, usually due to equipment limitation or simply because it is easier.

It is faster, easier and cheaper to take the time to randomize or split the experiment in blocks than having to repeat everything again!