DASware® design

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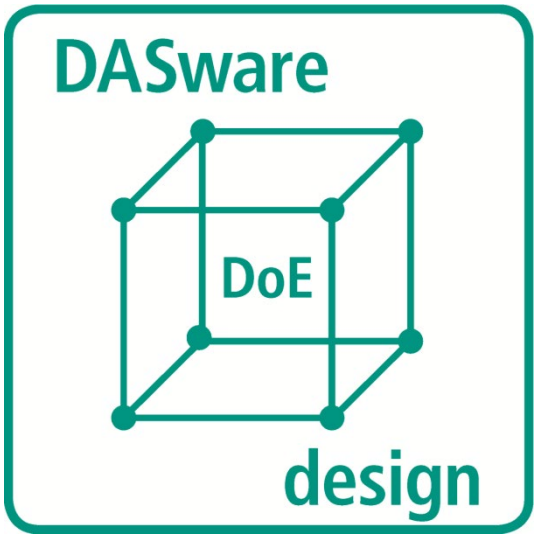
DASGIP TECHNOLOGY
an Eppendorf Company

User Manual



We know bioprocessing

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1. DASware® Solutions

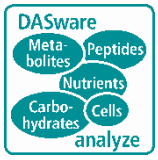
Increasing process complexity coupled with rising cost pressures and rapidly evolving regulatory requirements make today's process development efforts a special challenge. The pressure of achieving faster time to market for new and innovative biopharmaceuticals had led to the need to optimize every element of the total development workflow.

DASGIP *DASware* was designed as a suite of smart and flexible software solutions to accelerate bioprocess development. It enables interconnectivity of bioreactors with external labdevices, comprehensive data- and information management, factorial design of experiment and remote control of bioprocesses. *DASware* can be used with the *DASGIP Parallel Bioreactor System* for benchtop applications in microbiology and cell culture as well as with legacy benchtop bioreactor controllers.

In the past, bioreactor processing, recipe management, process and product analysis, data generation, data mining and storage were poorly interconnected thus requiring time-intensive manual work. *DASware* supports interconnectivity combined with sophisticated bioprocess information management. The utilization of *DASware* enables streamlined process development at benchtop scale in accordance with QbD standards.

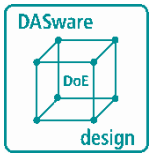
DASware consists of…

- for remote monitoring and control of bioprocesses



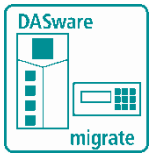
- for integration of lab devices with *DASGIP Parallel Bioreactor Systems*

- which facilitates a seamless interaction with legacy Corporate Historians and Process Control Systems



- to apply the Design of Experiments concept

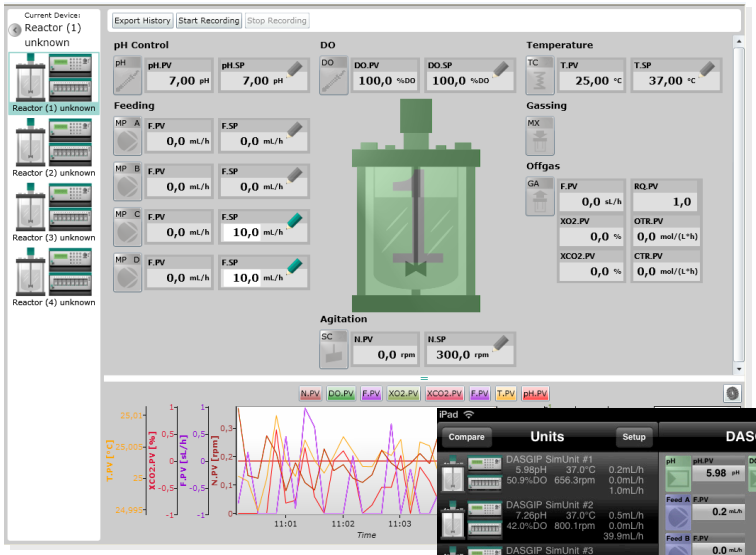
- a comprehensive and user-friendly data mining and information management solution for bioprocessing



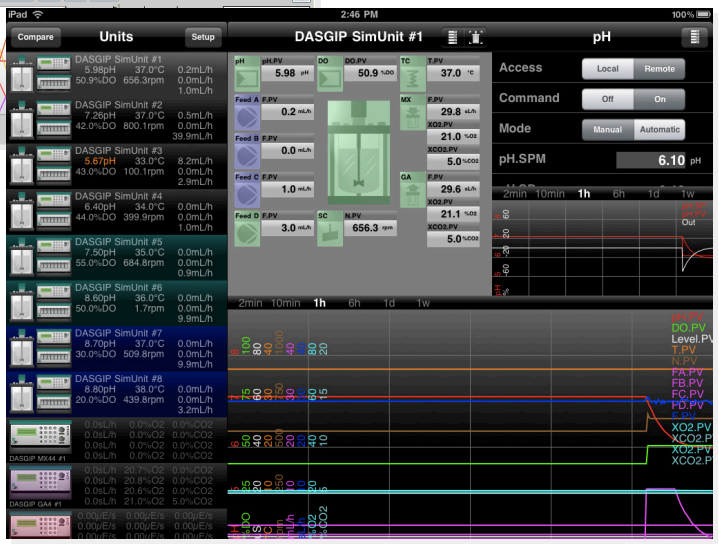
- to use all *DASware* solutions with legacy benchtop bioreactor control units

1.1. DASWARE® ACCESS

DASware access provides an unprecedented level of freedom and flexibility in the management of bioprocesses. Each *DASGIP Control System* on-site is accessible remotely by one or more remote clients simultaneously. Depending on the user-defined configuration and associated authentication either monitor or monitor and control access can be enabled for any network or mobile client.
Wi-Fi, Intranet, VPN and 4G connections can be used to provide web based access with almost every browser to one or more bioreactor systems via PC, Notebook or Netbook. The unique *DASGIP iApp* supports access from iPhone, iPod touch and iPad, optionally with webcam support.



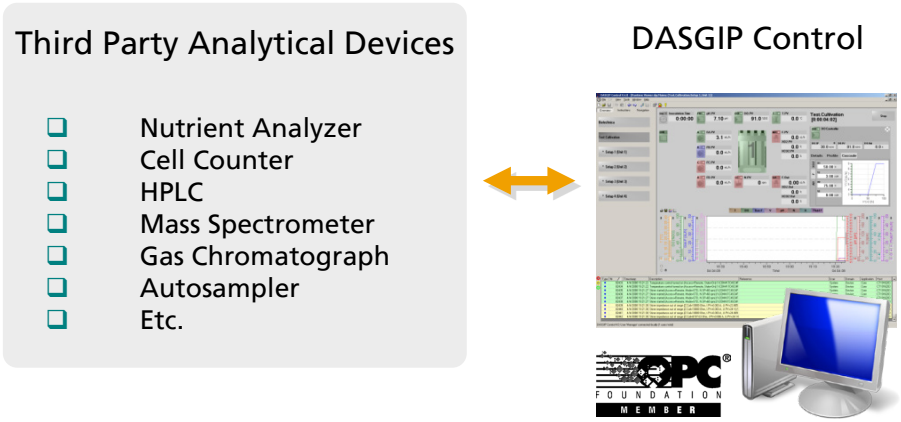
DASware access



DASGIP iApp

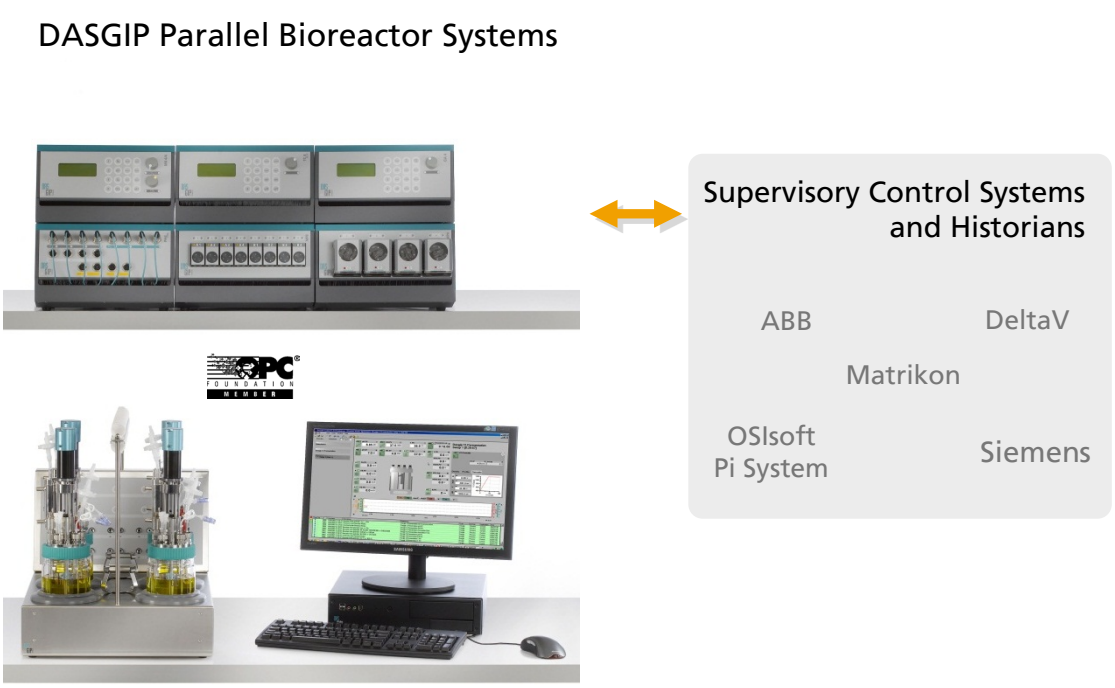
1.2. DASWARE® ANALYZE

DASware analyze was designed for seamless integration of sampling and analytical laboratory devices to the bioreactor system. A broad and growing range of analyzers can be integrated, among them nutrient analyzers, cell counter (YSI Life Science, Nova Biomedical), automation platform and autosampler (Bayer Technology and Services) biomass monitors (Aber Instruments), HPLC (Waters, Shimadzu) and mass spectrometers (Thermo Scientific).
The OPC network protocol allows for interconnectivity between the bioreactor system and the analyzer, including the possibility of direct feedback from the bioreactor system according to online measured analytical data. This facilitates feedback control loops for e.g. nutrients, biomass or product concentrations. Online calculations as well as event and data-driven decisions are supported. The unique bidirectional OPC communication, available for supporting devices enables sampling on demand and process dependent analyzer panel selection.



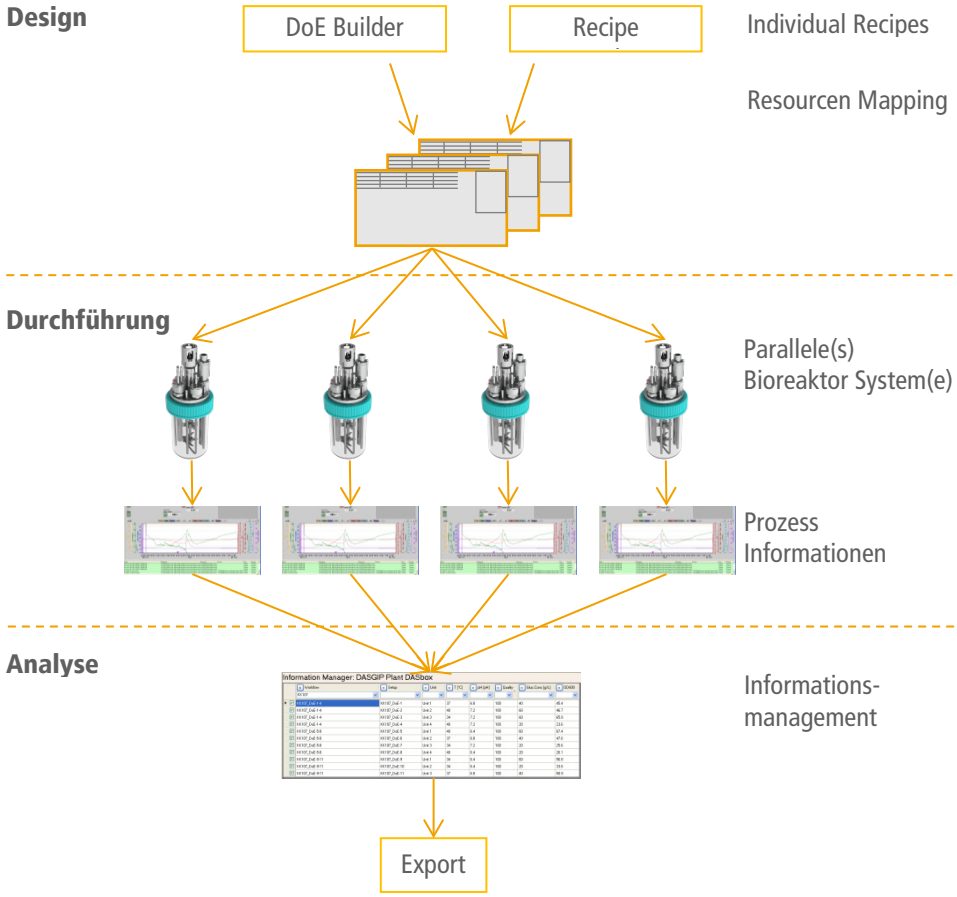
1.3. DASWARE® CONNECT

DASware connect was designed to integrate DASGIP's Parallel Bioreactor Systems and 3rd party bioreactor controllers into process control systems and legacy corporate historians. This includes but is not limited to Emerson DeltaV®, Siemens SIMATIC PCS 7, ABB 800 xA, OSIsoft PI System® and Matrikon OPC Historian. *DASware connect* facilitates company-wide access to all relevant bioprocess data like set-points, process values, feed-profiles, calibration and controller parameters as well as events and alarms.



1.4. DASWARE® DESIGN

DASGIP's bioreactor systems serve as an ideal platform to carry out DoE on bioreactors in parallel. *DASware* design automatically compiles DoE information from 3rd party DoE tools into recipes and feeds-back response information into DoE and multivariate analysis and reporting tools.



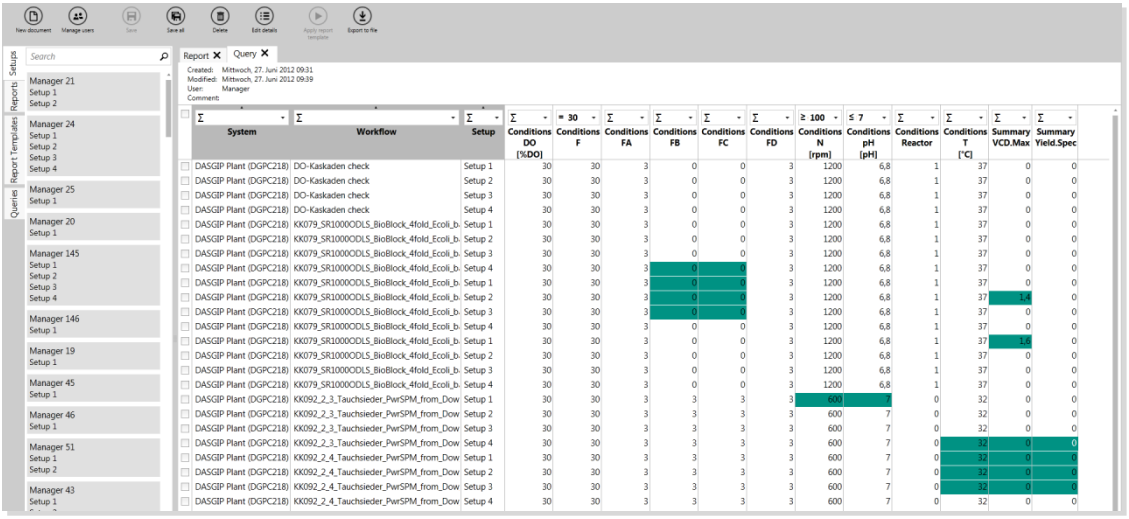
DASware design comes with a Full Factorial DoE builder. Alternatively, a large variety of DoE designs for screening, process development and optimization can be automatically imported from most powerful 3rd party DoE tools like JMP and others. Parallel recipes incorporating the DoE factor variations (i.e. pH, DO, T set points or feed rates) are automatically populated. Following DASGIP's Point-Click-Grow concept, they can be carried out on a set of bioreactors with a single mouse-click. DoE response information is collected and prepared for an automated export.

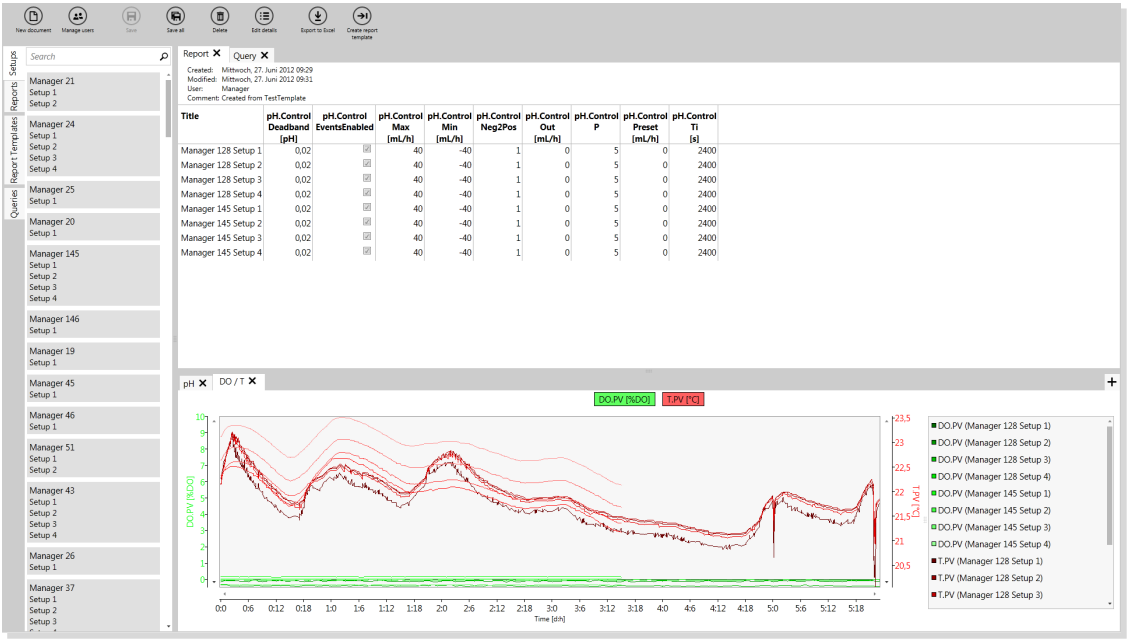
1.5. DASWARE® DISCOVER

DASware discover helps you to structure efficiently the assortment of process information like

- Process values
- Process events
- Recipes
- Biological,
- Chemical and
- Analytical information

Use this intuitive database query tool to initiate real-time retrievals and run-to-run comparisons. All critical process information is clearly listed as table and can be configured. In addition, you can easily generate charts in Microsoft Excel® using *DASGIP Documentation Wizard*.





1.6. DASWARE® MIGRATE

A significant installed base of ageing bioreactor controllers offers rudimentary cultivation control capabilities. However, recent advances such as bioprocess data management, analytical device integration, DoE support and remote control, available from DASware solutions, are often missing. Now users of 3rd party bioreactor controllers can also benefit from DASGIP's intelligent software solutions.

DASware migrate integrates most common 3rd party bioreactor control units and systems (e.g. Satorius Stedim Biotech & Applikon) as well as DASGIP modules into legacy bioreactor controllers (e.g. Off-gas analyzer GA4, Gas-mixing station MX4/4, Mulitipump MP8).

Bioprocess Control Systems ⟷ DASGIP Control

DASware migrate enables

- Access to the complete DASware solution suite
- Sharing recipes between different bioreactor controllers
- Powerful Microsoft Excel® reporting
- Comprehensive information management and process data historian
- Analytical lab device integration
- DoE and remote control

2. Introduction

In current bioprocessing technologies, process-oriented criteria are playing an increasingly important role in the assessment of product quality. Quality by Design (QbD) is a concept that was highlighted, among others, in an FDA guideline on process validation (General Principles and Practices, FDA, January 2011). It states the need for monitoring procedures already at the development stage in order to achieve a controlled process and to ensure a defined product quality.

This chapter outlines the concepts of design space, design of experiments (DoE) and DoE fields of application. This is followed by a brief overview of the parallel bioreactor system *DASbox* and *DASware design* itself, including a schematic process flow.

Design space

In general, different process settings can be used to achieve good product results. This multidimensional space which is defined by the factors and the corresponding levels is referred to as design space (Fig 1). Within this space, the required quality of the product is guaranteed.

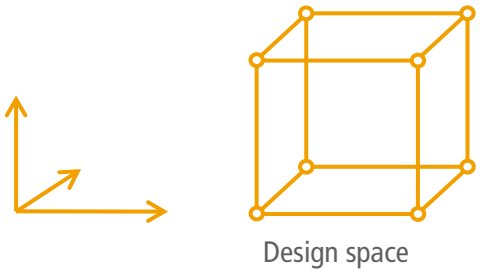


Fig 1: Design space with 3 factors in 2 levels

The identification of a suitable design space requires a variety of implementations. Design of experiments (DOE) can be helpful to reduce the extent of required testing – the total number of individual experiments.

Design of experiments (DoE)

DoE (Fig. 2) is often used to reduce the number of process runs necessary to determine a suitable design space (Fig 1) for the manufacture of a product. The amount of time saved by using a multifactorial DoE approach, compared to the classical variation of individual parameters (one factor at a time), significantly reduces the development overhead and helps to shorten development times.

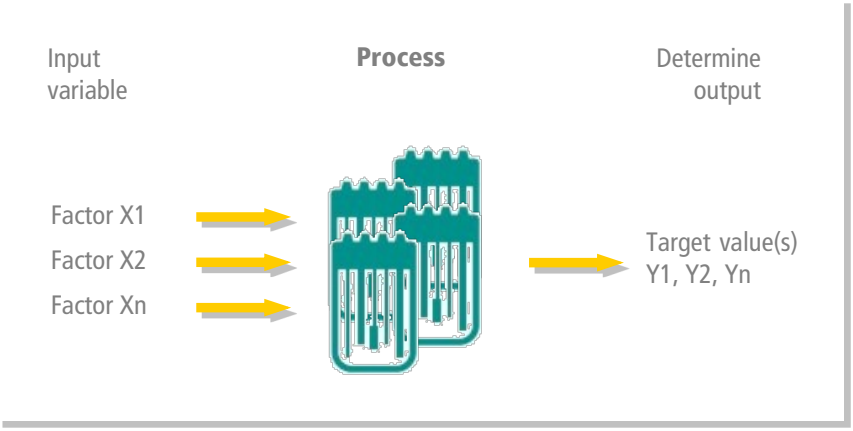


Fig. 2: DoE scheme

The advantages of DoE include:

- Fewer individual experiments required.
- Main effects and interactions are recorded.
- Effects of main factors can be quantified and statistically evaluated.
- Process knowledge is improved, allowing the development of more efficient procedures.
- The description of functional process connections facilitates the definition of a mathematical model and thus the design space (Fig 1).

The following points are to be noted regarding DoE-based process development:

- The factors which have an influence on the target value(s) must be identified.
- The process settings must be varied meaningfully, e.g., within the physiologically acceptable temperature or pH range of the used microorganisms/cells.
- Discrete factor values can be selected using prior knowledge or previous examinations.

DoE Fields of Application

A DoE is used, for example, in the process development of biopharmaceuticals, such as vaccines. Equally suitable is the production of biochemicals such as bioethanol or also foodstuffs such as lactic acid. Individual fields of application for optimization are screening, strain or cell line characterization as well as process development itself. The following figure (Fig. 3) provides a schematic overview of the DoE fields of application.

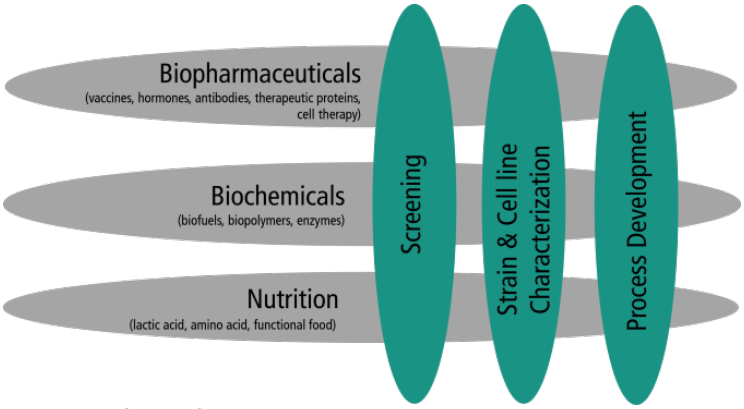


Fig. 3: DoE fields of application

Since DoE minimizes the number of process runs and optimizes the acquisition of knowledge, a number of software products for DoE is available in the market. The following is a list of some well-known products:

- JMP®
- Design Expert®
- Minitab™
- MODDE®

System Platform for DoE

Since in the field of bioprocess applications typical designs of experiments can be carried out at the same time using a number of bioreactors, a parallel system is perfectly suited to support the principle of DoE.

DASGIP Parallel Bioreactor Systems ensure defined and controlled process conditions to facilitate both the screening of bacteria or cell cultures and the optimization of media or substrate quantities on a small scale. For example, the easily extendable modular *DASbox* System (four mini bioreactors per unit) offers controlled and reproducible cultivation results.

In *DASGIP Control* all processes can be precisely defined, optimized and adapted. In addition, all results are accurately and precisely documented.

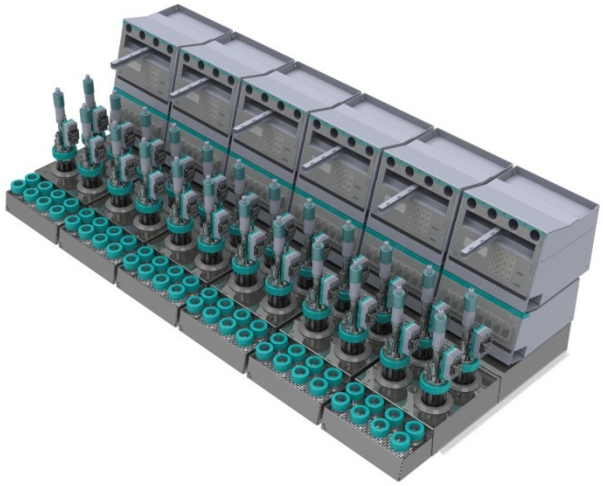


Fig. 4: *DASbox* System with 6 x 4 bioreactor units

DASware design

DASware design combines statistical design of experiments (DOE) with parallel bioreactor systems, e.g., the *DASbox* or other modular parallel bioreactor systems from DASGIP. The degree of automation achieved considerably simplifies and shortens the processes.

The following is a schematic outline of an experimental sequence (Fig 5).

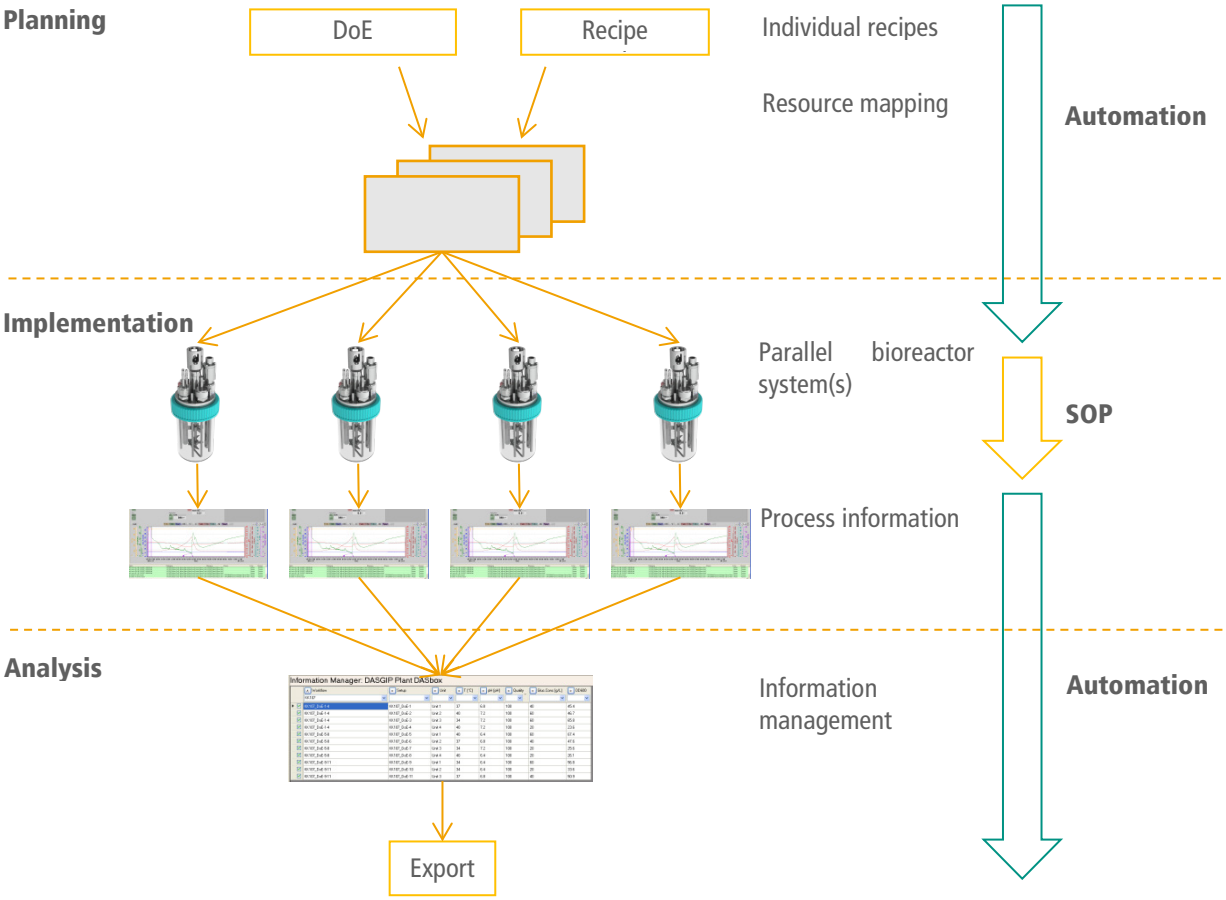


Fig 5: Schematic process flow

- Within *DASware design*, the DoE Builder facilitates the input of all DoE-relevant parameters. There is also an option to directly integrate files from DoE software tools.
- Individual recipes can be used and resources can be assigned.
- A generated workflow can be saved with one click and seamlessly integrated into *DASGIP Control* via the Recipe Editor. Parallel and controlled process implementation is carried out here, if desired, according to a SOP.
- All process information is automatically saved and can be analyzed and supplemented directly in *DASGIP Control* using the *DASGIP Information Manager*.
- Afterwards, export options allow the process information gained to be traced in the statistical analysis software. Improved process knowledge enables the development of more efficient procedures.

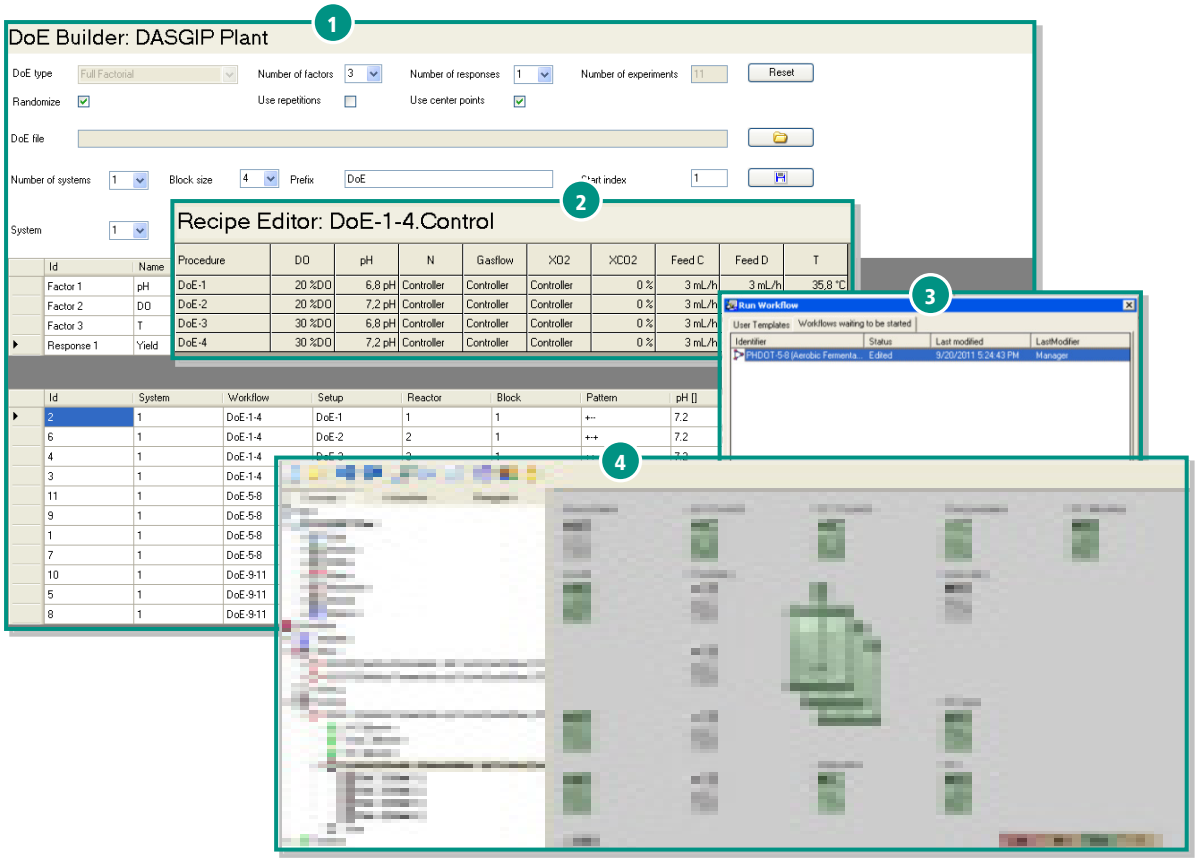
3. DASware® design

The configuration, implementation and evaluation of complex experimental designs is a major challenge in process development. *DASware design* helps implementing such designs by seamlessly integrating DoE in *DASGIP Control* and using its precise, parallel process implementation.

With DoE the most important process parameters are initially identified to enable optimal process settings to be made. These parameters can be easily entered via the DoE Builder in *DASware design*. All values are then seamlessly transferred to the Recipe Editor, where they can be supplemented. After a workflow has been started, the parallel bioreactor processes are carried out under approved controlled conditions. Once this is completed, the process results are documented and can be easily evaluated using the *DASGIP Information Manager*. In addition, the evaluated data can be reimported into to a standard DoE software tool.

The following figure summarizes the sequence of a DoE-based process in *DASGIP Control* using *DASware design*. It also provides an overview of the structure of this chapter.

1. DoE Builder (3.1)
2. Recipe Editor (3.2)
3. Run workflow (3.3)
4. Monitoring and control of the cultivation processes in bioreactors (3.4)



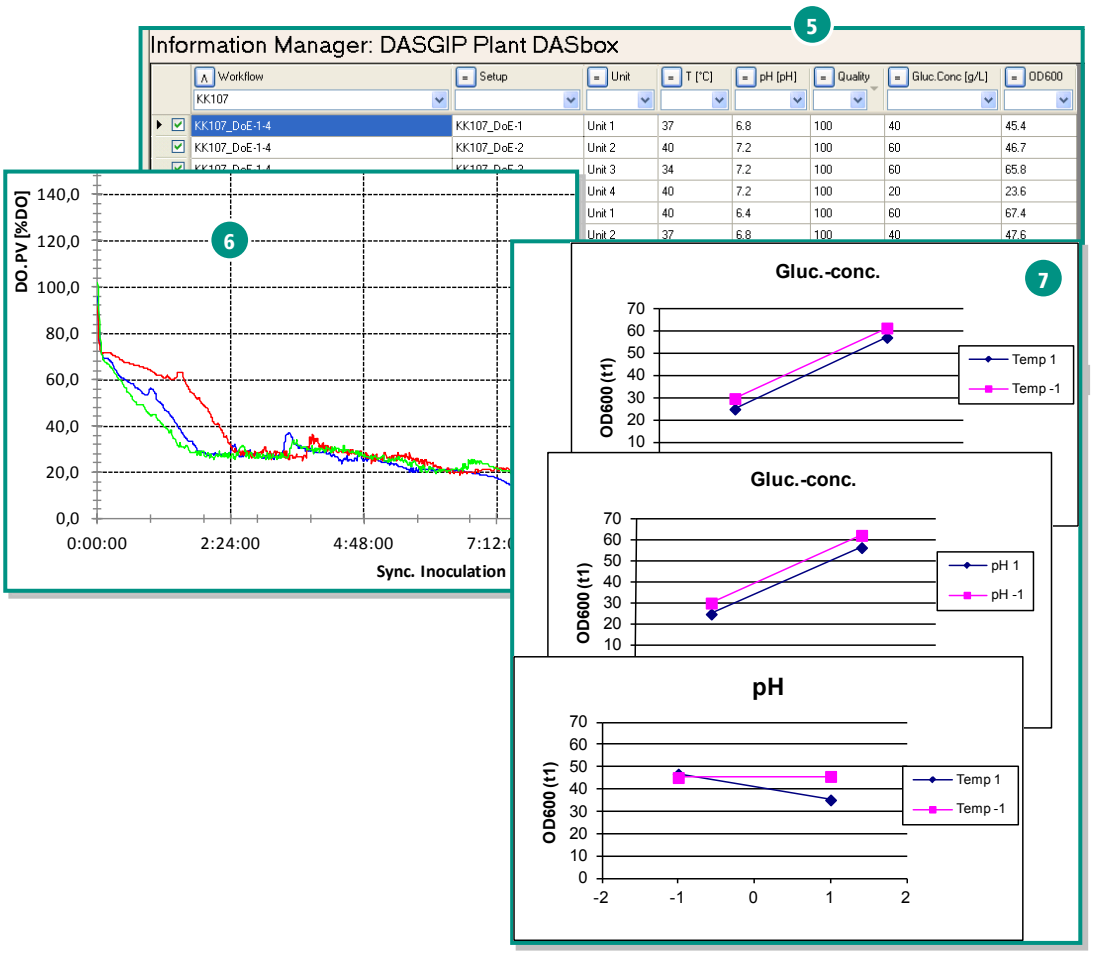


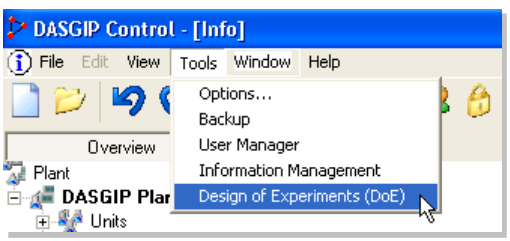
Fig. 6: Overview of a DoE-based process flow with *DASware design*

5. Documentation using the Information Manager (3.5)
6. Analysis of historical cultivation data for the plausibility check in the *DASGIP Documentation Wizard* (3.6)
7. DoE evaluation (4)

3.1. DoE Builder

The DoE Builder allows the direct selection or input of all DoE-relevant parameters.

Click on Tools in the *DASGIP Control* menu bar to open the DoE Builder by clicking on Design of Experiments (DoE).



Structure of the DoE Builder:

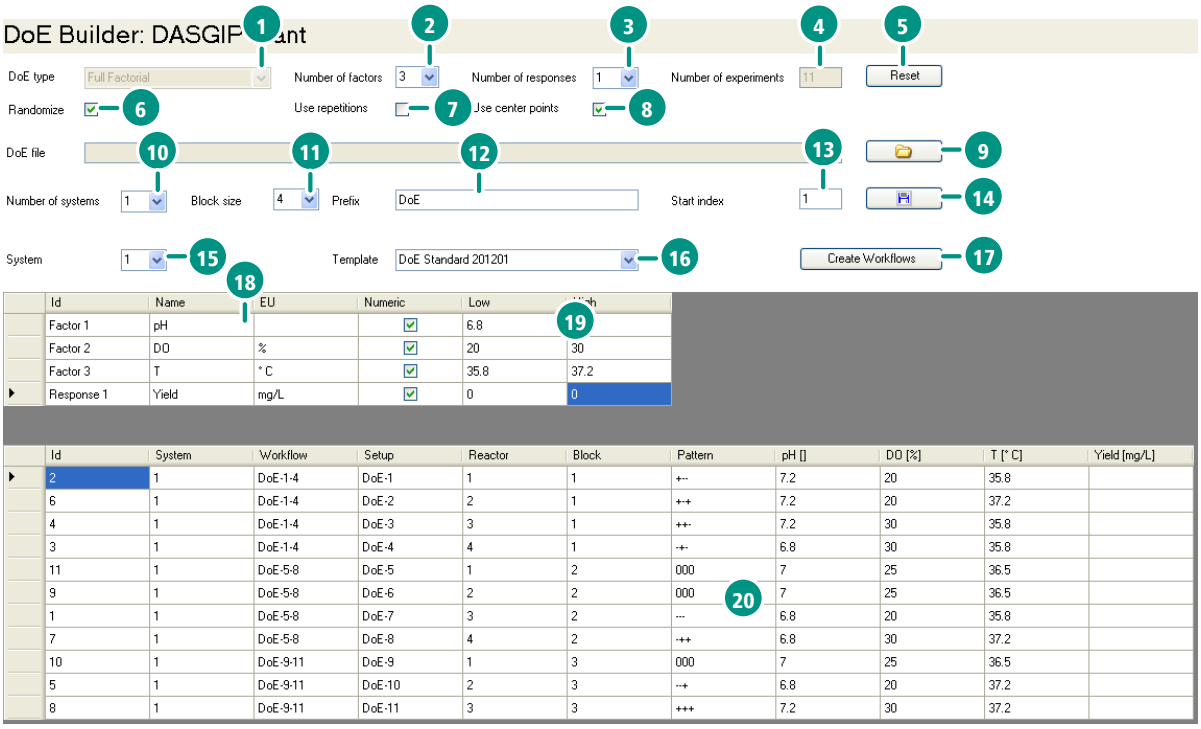


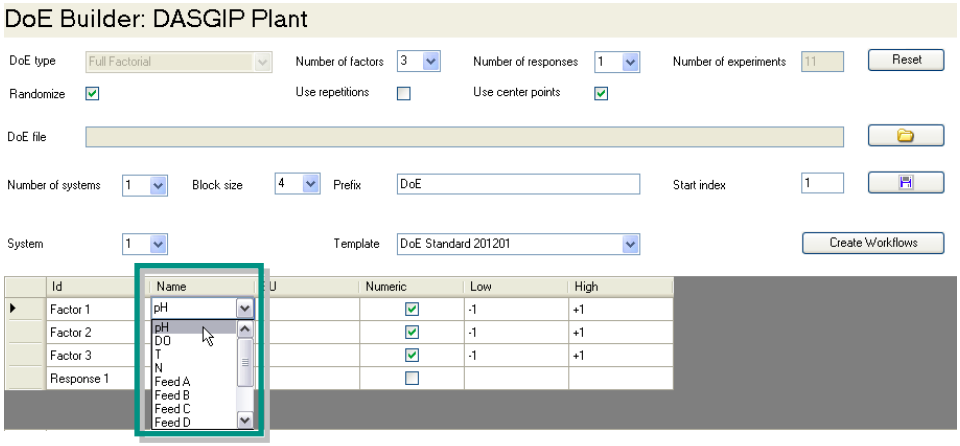
Fig. 7: Overview of the DoE Builder

No.	Control element	Description
1.	DoE type	For selecting a DoE type (e.g., Full Factorial).
2.	Number of factors	Selects the number of factors
3.	Number of responses	Selects the number of responses
4.	Number of experiments	Indicates the number of individual experiments
5.	Reset	Resets all entries.
6.	Randomize	If you select this checkbox, a random assignment of individual experiments takes place to the existing bioreactors.
7.	Use repetitions	If you select this checkbox, all experiment runs are automatically doubled.
8.	Use center points	Here you can select the addition of a center point identification per reactor unit.
9.	Open	Imports DoE data see 4.1.2.
10.	Number of systems	For selecting the number of available systems see also 3.1.1.
11.	Block size	Indicates the number of bioreactors per unit/block
12.	Prefix	Here you can enter a prefix to be attached to the front of all workflow and setup names.
13.	Start index	Adjusts the numbering start point.
14.	Save	Saves all entries see 3.1.3.
15.	System	Limits the selection by system.
16.	Template	Selects previously created recipe templates.
17.	Creating workflows	Uses the entered DoE data to automatically compile executable workflows for *DASGIP Control* see 3.1.4.
18.	Name	For defining factor and response names.
19.	Low / High	Level assignment for factors.
20.	Table	The DoE table is automatically adjusted and filled.

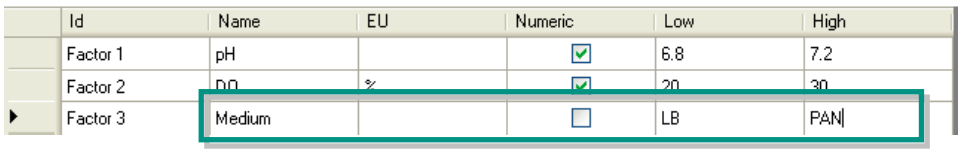
3.1.1. Selecting or Entering Factors

The number of table rows in the lower part of the DoE Builder is adapted to the number of factors and the response(s) that were selected.

Standard factor names that correspond to the *DASGIP Control* naming convention can be selected in the dropdown menu in the Name column. The EU (unit), Low and High (level) columns are then automatically filled with default values. You can click on these values and adjust them as necessary.



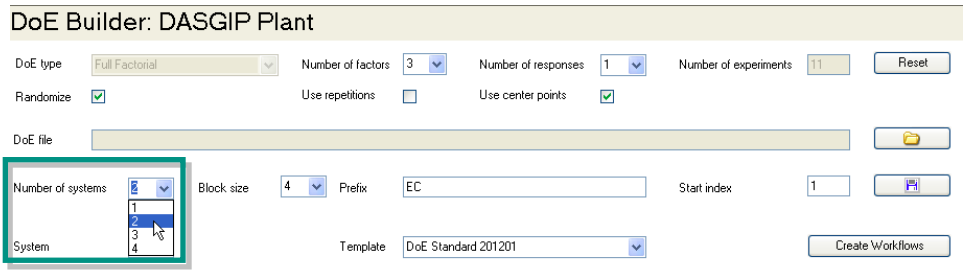
You can also directly enter new factors which have not yet been stored.



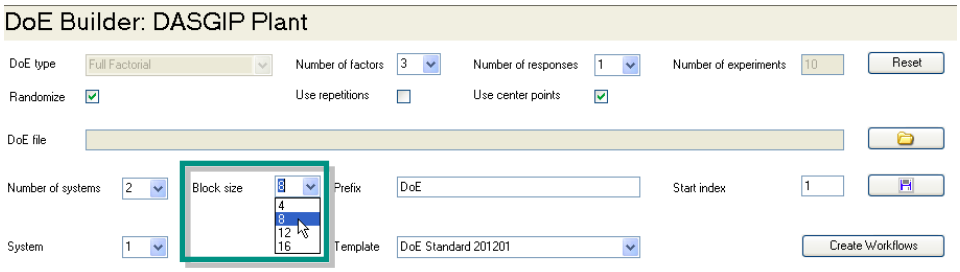
3.1.2. Selecting Resources

The middle part of the DoE Builder allows the selection and assignment of existing resources.

Select the number of used systems in the dropdown menu next to Number of systems. If several systems exist, you can select which system should be used via System.



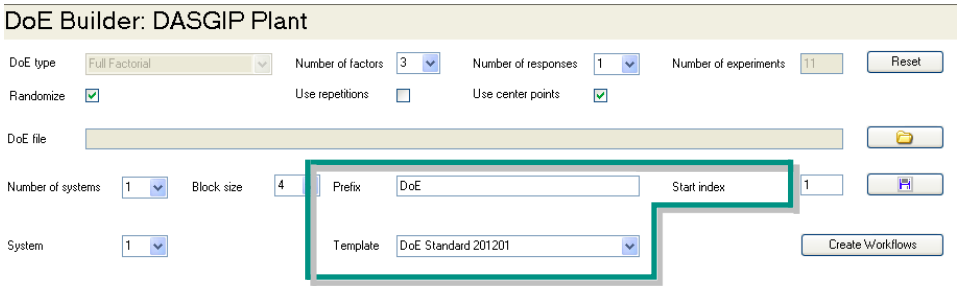
Also select the number of reactors of a system unit next to Block size.



Enter a prefix to be attached to the front of all workflow and setup entries.

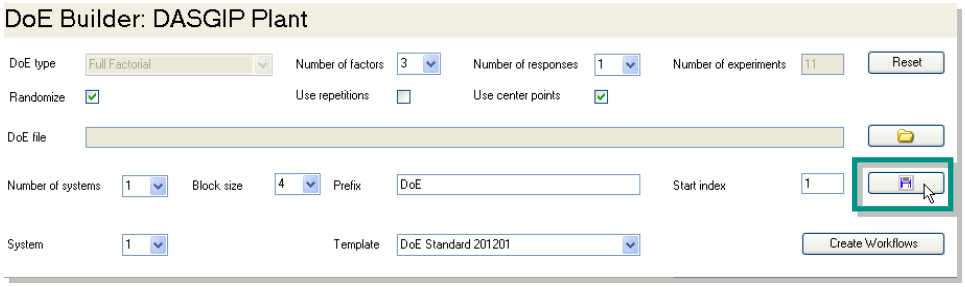
In the Template dropdown, select previously saved template files that contain all experiment-relevant parameters (e.g., speed, temperature, feed rate etc.).

Next to Start index enter the number at which the setup numbering should start. It may be useful to adjust this number if, e.g., different systems are used.



3.1.3. Saving DoE Settings

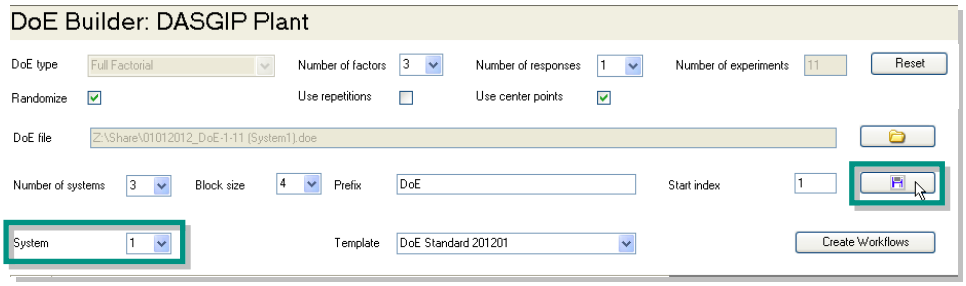
To save all the settings you have made, click on the Save button. A file manager dialog appears in which you can enter a file name and select a storage location.



Resource Example

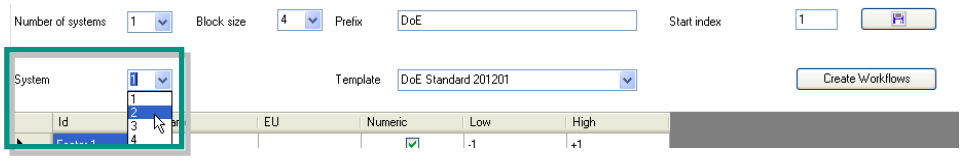
The use of three systems with a block size of four reactors is shown as an example.

1. 3 is selected for Number of systems and 4 for Block size. To save the settings for the first system, number 1 has to be selected for System.



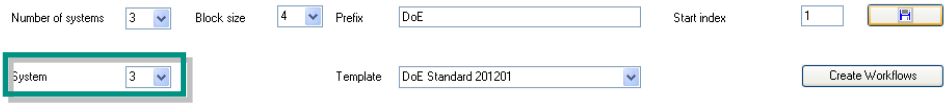
The saved .doe file can be reimported at the desired process computer in the DoE Builder using the  button.

2. To save the entries for the second system, number 2 has to be selected for System.



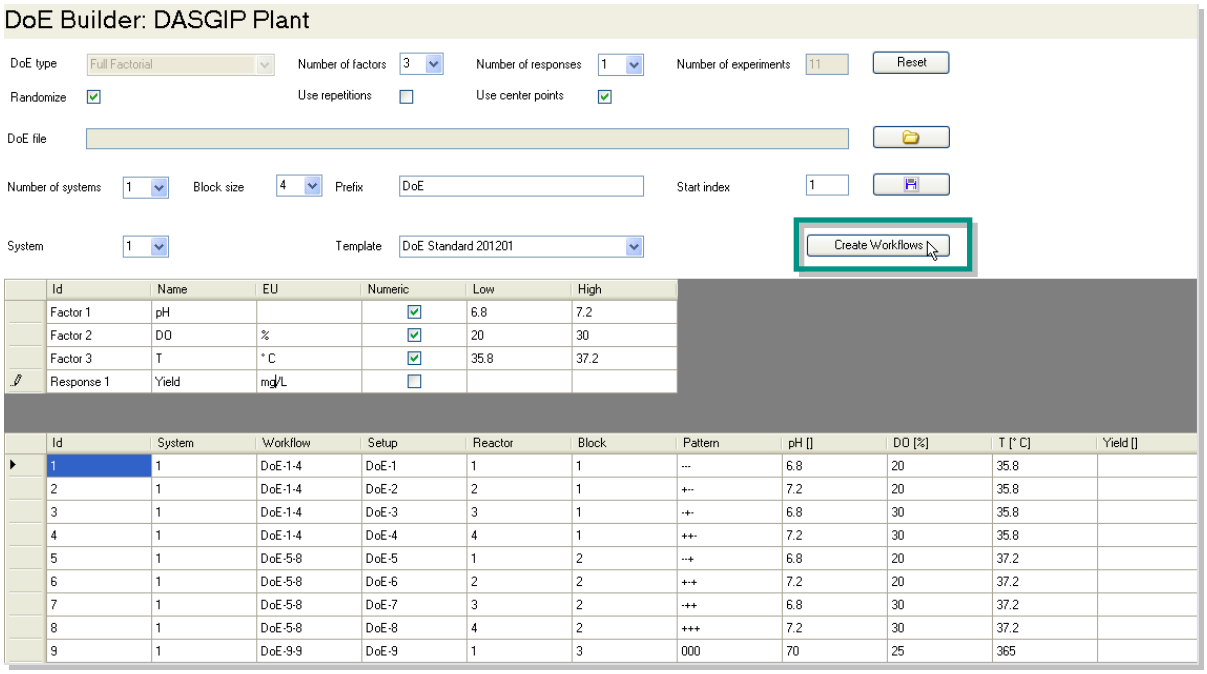
This ensures that only the data relevant to the second system is saved and can be transferred to a process computer.

3. The procedure with the data for the third system is identical. In this case, number 3 has to be selected for System before saving.



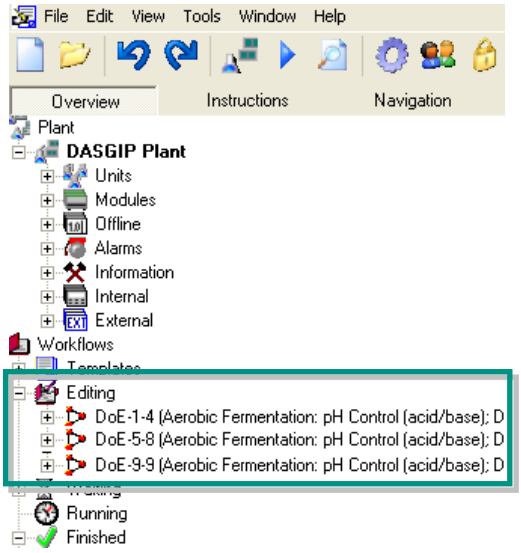
3.1.4. Create Workflows

After all DoE parameters have been entered, which are summarized in a table in the lower part of the DoE Builder, they are merged into the selected recipe template using the Create Workflows button.



This ensures that several workflows are automatically created, e.g., if several systems are used, or a single system with a low block size is used several times. Each workflow contains a set of individual recipes containing the parameter variations of the design.

All automatically created workflows appear in *DASGIP Control* at the left under Editing in the Navigation Tree.



Before carrying out the experiment, it is recommended that you check the individual entries in the Recipe Editor (3.2) and complement them as necessary.

The workflows from the example are divided as follows:

System 1

Recipe Editor: DoE-1-4.Control

Procedure	DO	pH	N	Gasflow	XO2	XCO2	Feed C	Feed D	T
DoE-1	30 %DO	6.4 pH	Controller	Controller	Controller	0 %	0 mL/h		37 °C
DoE-2	30 %DO	7.2 pH	Controller	Controller	Controller	0 %	3 mL/h		37 °C
DoE-3	30 %DO	7.2 pH	Controller	Controller	Controller	0 %	3 mL/h		37 °C
DoE-4	30 %DO	6.4 pH	Controller	Controller	Controller	0 %	3 mL/h		34 °C

System 2

Recipe Editor: DoE-5-8.Control

Procedure	DO	pH	N	Gasflow	XO2	XCO2	Feed C	Feed D	T
DoE-5	30 %DO	6.8 pH	Controller	Controller	Controller	0 %	3 mL/h		35.5 °C
DoE-6	30 %DO	6.8 pH	Controller	Controller	Controller	0 %	3 mL/h		35.5 °C
DoE-7	30 %DO	7.2 pH	Controller	Controller	Controller	0 %	3 mL/h		37 °C
DoE-8	30 %DO	7.2 pH	Controller	Controller	Controller	0 %	3 mL/h		34 °C

System 3

Recipe Editor: DoE-9-11.Control

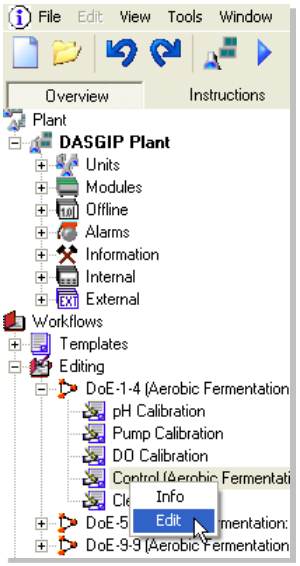
Procedure	DO	pH	N	Gasflow	XO2	XCO2	Feed C	Feed D	T
DoE-9	30 %DO	6.4 pH	Controller	Controller	Controller	0 %	3 mL/h		37 °C
DoE-10	30 %DO	6.8 pH	Controller	Controller	Controller	0 %	3 mL/h		35.5 °C
DoE-11	30 %DO	6.8 pH	Controller	Controller	Controller	0 %	3 mL/h		34 °C

3.2. Recipe Editor

The Recipe Editor provides an intuitive interface representing all parameter variations of all setups in a table. In the recipe editor any individual parameter adjustment is possible before starting the process.

3.2.1. Open

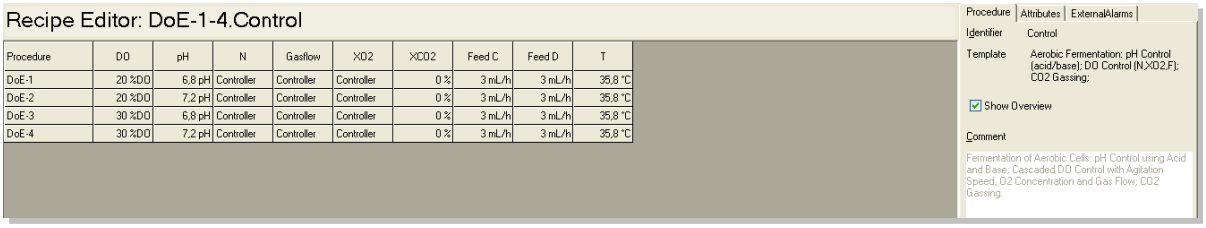
By right-clicking on the name of the main procedure (Control…), a context menu appears in which you can click on Edit in order to open the Recipe Editor of *DASGIP Control*.



3.2.2. Overview

During the experimental setup, in addition to the preconfigured DoE parameters, other process parameters like PID or feed-rates can be adjusted either all descriptive key parameters can be entered at this point. This includes details on the used strain, cell line, medium composition, temperature etc. The Recipe Editor allows these entries to be made individually for each setup/reactor.

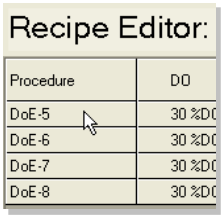
The appearance of the editor varies depending on the configuration of each *DASGIP Control System* and according to the defined workflow. The table displayed contains different columns and one row for each reactor (setup).

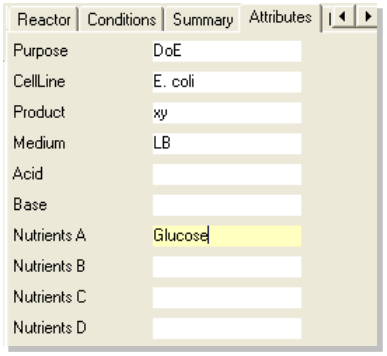


Comprehensive, detailed information on processes can be especially useful for the individual comparison of historical process data.

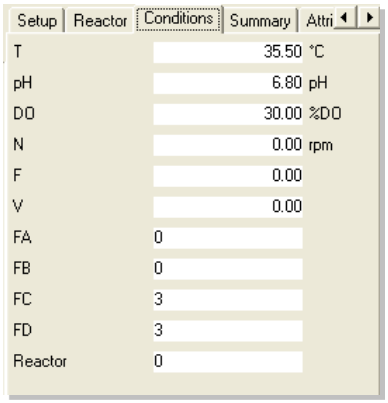
Attributes and conditions for individual setups can be easily entered into predefined entry fields. The fields which appear depend on the previous configuration in the Workflow Editor (for more information see User Manual *DASware migrate* 2.3.2.).

Clicking on a single setup cell displays the corresponding buttons (Attributes, Conditions) in the right area of the editor. Information can be added here.



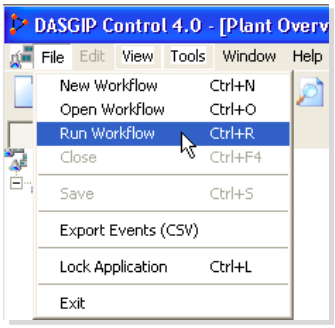


When a workflow has been created from the DoE Builder via Create Workflow and the usual factor names (e.g., pH, DO, T etc.) have been selected in *DASGIP Control*, the Recipe Editor is automatically filled with the parameters.

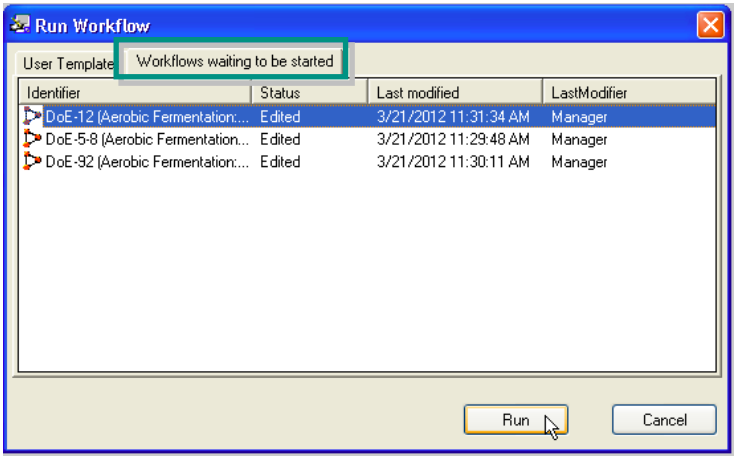


3.3. STARTING A WORKFLOW [RUN]

To start a workflow created in the Workflow Editor, you can either click on Run Workflow under File in the menu bar or click directly on the corresponding button in the toolbar.



The following dialog lists the workflows that have not yet been started under Workflows waiting to be started.



Click on the Run button to continue the process.

3.4. Process Monitoring and Control

The selected procedures are started depending on workflow.

After the main procedure has started, the Summary View is displayed. It summarizes all the important process operations at a glance.

A Summary View is shown below which is based on a DoE experiment setup with varied temperature set points (Fig. 8). By clicking on the temperature symbol, the values of the temperature controller are displayed on the right side. The diagram in the lower part represents the course of the dissolved oxygen (DO) in the medium, in addition to the three different temperature set points.

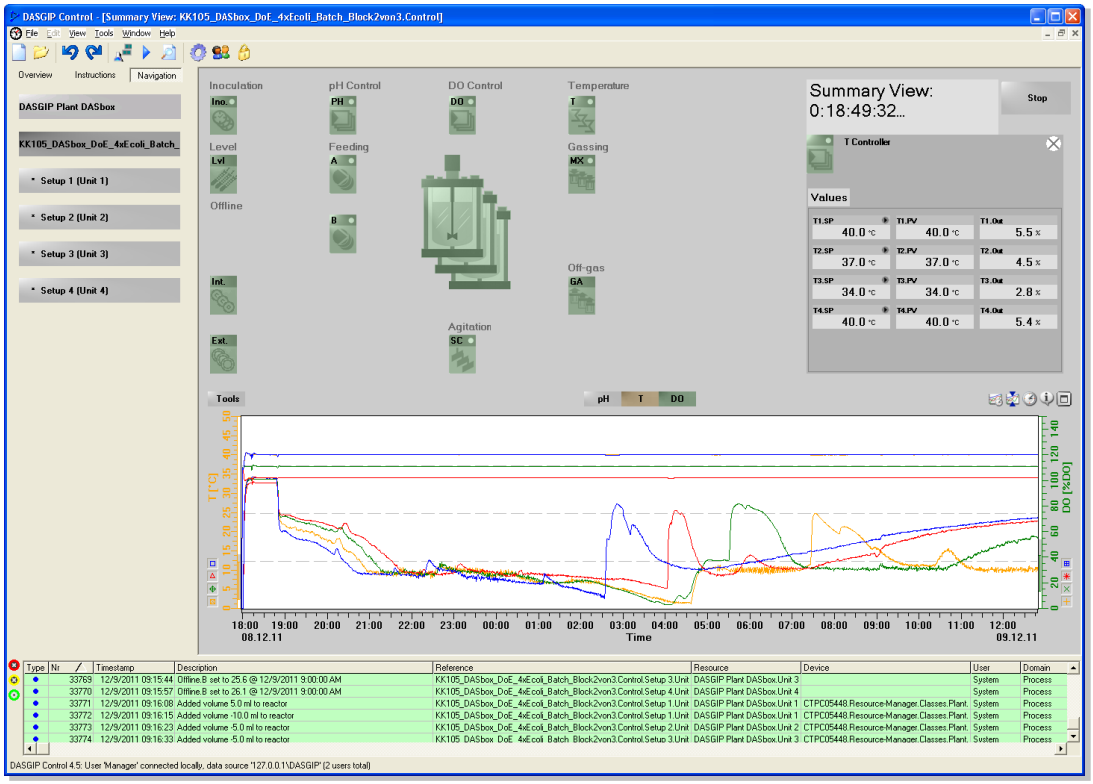


Fig. 8: Exemplary overview (Summary View) in *DASGIP Control*, bioreactor processes of a variation of temperature set points

More information can be found in the *DASGIP Control* User Manual.

After completion of all processes, the process data is saved in *DASGIP Control* and can be used for further analysis.

3.5. DASGIP Information Manager

In addition to the automatic documentation of all general process and DoE-relevant data, it is also possible to supplement additional user-definable information such as the product yield, the maximum density of vital cells (VCD.Max) or general quality data. This information is entered in the *DASGIP Information Manager*. Such data can contain information on the DoE responses to be exported in an analysis tool.

The following figure provides an overview of the *DASGIP Information Manager*.

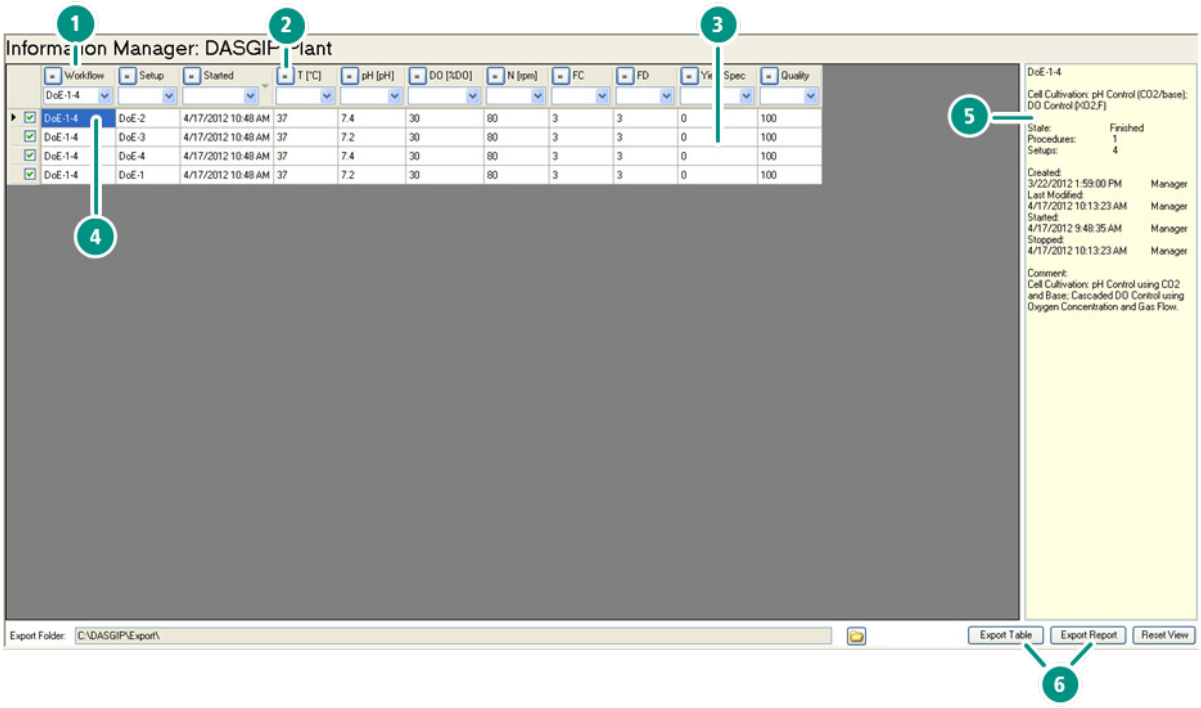


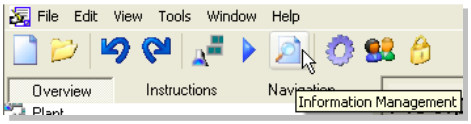
Fig. 9: Overview of the *DASGIP Information Manager*

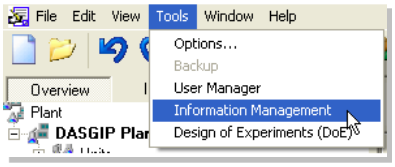
1. Query criteria appear in the column headings.
2. You can use the buttons to select comparative operators in order to limit a query.
3. It is possible to subsequently enter values, such as Yield or Quality, that have been determined based on process data. (Additional individual columns can be used if they were defined before the start of the process. More information can be found in the DASGIP Control Manual).
4. The query results are summarized in table form and can be selected individually.
5. General data on selected setups or workflows is displayed.
6. Export options.

In the *DASGIP Information Manager* it is also possible to carry out queries during an ongoing experiment. However, note that the current data of this ongoing experiment cannot yet be included in the query. This data will only be available after the experiment has been completed.

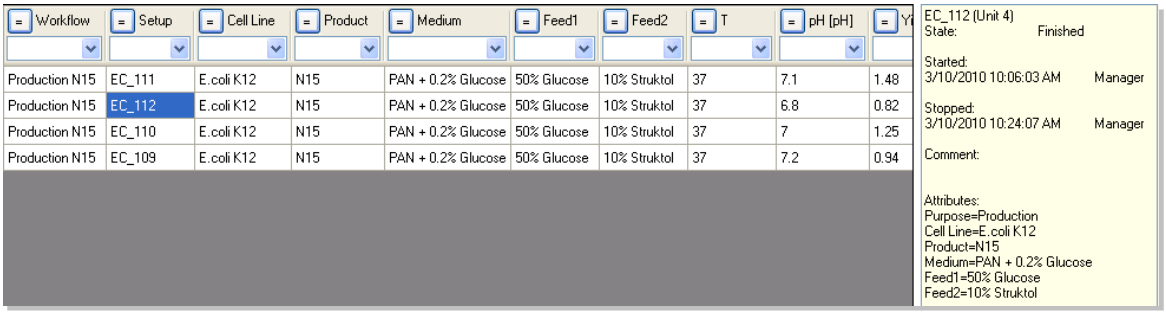
A query in the *DASGIP Information Manager* includes all the experiment runs with the corresponding recipes and process data as well as the events and user-specific context information. Only the procedure data of calibrations, CIP and Clean is not included. The result of a query can then be exported to Microsoft Excel®.

1. You can either click directly on the Information Management button in the taskbar to open the *DASGIP Information Manager* or call the Tools menu and select the Information Management menu item.



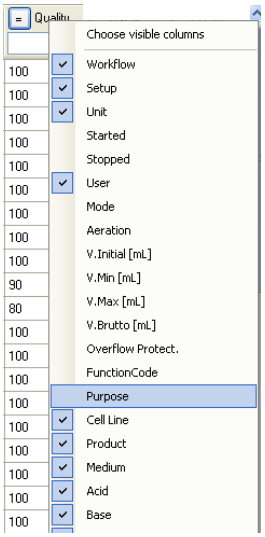


2. When you click on a corresponding cell, general information on a workflow or setup is displayed on the right.

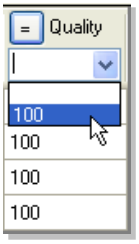


All entries can be edited, with the exception of the workflow, setup and unit names. Please use this option with caution.

3. All available query criteria are displayed when you click with the right mouse button on a column heading. From the dropdown menu, select the column headings to be displayed. This allows the displayed data to be limited as needed and saved.

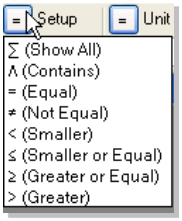


4. You can also directly enter query values below each column heading in the input field or select values from the dropdown list which is automatically filled with existing values.



The additional entries for this process are saved together with the process values and are available for additional analysis in each further export.

5. You can use the button to the left next to each column heading to select relational operators for the qualification of a query.



Example: You want to select all process data where *E. coli* K12 was used and for which a product yield of at least 0.2 g/ L was achieved. For this, the two query criteria are entered in the corresponding CellLine and Product Yield [g/L] columns. The table will then list all the matching process data.

CellLine	Medium	Feed1	Feed2	T [°C]	pH [pH]	DO [%DO]	Yield.Spec [pg/Cell/d]	Quality [%]	Product.Yield [g/L]	CDW [g/L]
E. coli K12	PAN + 0,2% Gluc	50% Glucose	10% Struktol	37.0	6.50	30	1.500	90	1.245	90.0
E. coli K12	PAN + 0,2% Gluc	50% Glucose	10% Struktol	37.0	7.00	30	0.850	40	0.214	45.0
E. coli K12	PAN + 0,2% Gluc	50% Glucose	10% Struktol	37.0	5.50	30	1.300	90	0.985	80.0
E. coli K12	PAN + 0,2% Gluc	50% Glucose	10% Struktol	37.0	6.00	30	1.400	85	0.758	75.0

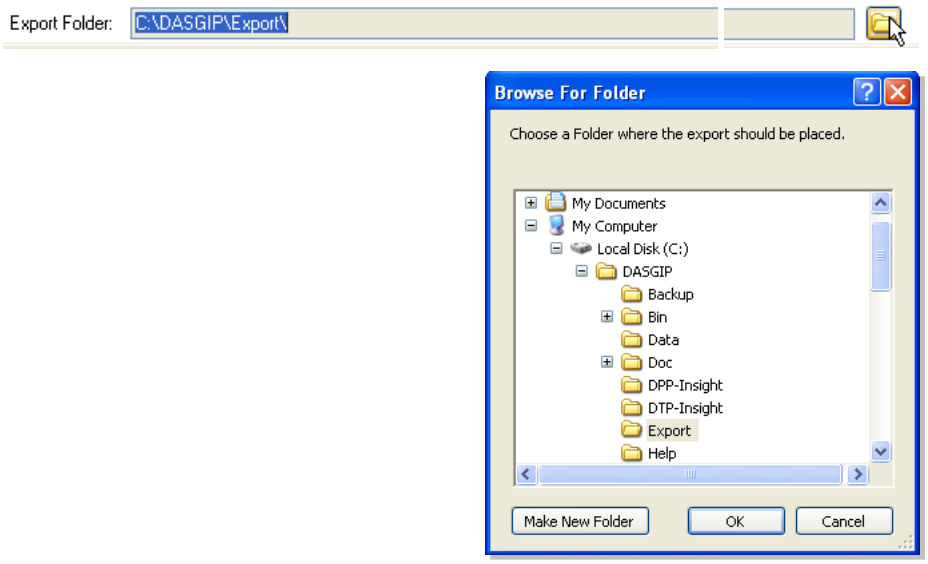
The entry of search criteria is not case-sensitive, but spaces are considered.

With the help of historical data you can, for example, also carry out a plausibility check of new process runs using a batch to batch comparison. Selecting actual and historical runs at the same time generates a combined report for subsequent independent analysis.

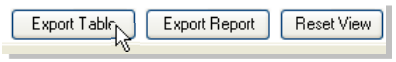
Exporting Data

You can export all query data using the Export Table or Export Report buttons (including all process data).

In addition to Export Folder:, the storage path of the export file is displayed. This can be changed using the Folder button in the Browse For Folder dialog window.



The compiled information that is listed in table form can be exported in various forms:



- Select Export Table in order to export all DoE-relevant parameters as a .txt file.

  An exported DoE table is shown below as an example. It is used as a basis for further analysis in Microsoft Excel® or special DoE analysis tools, such as JMP.

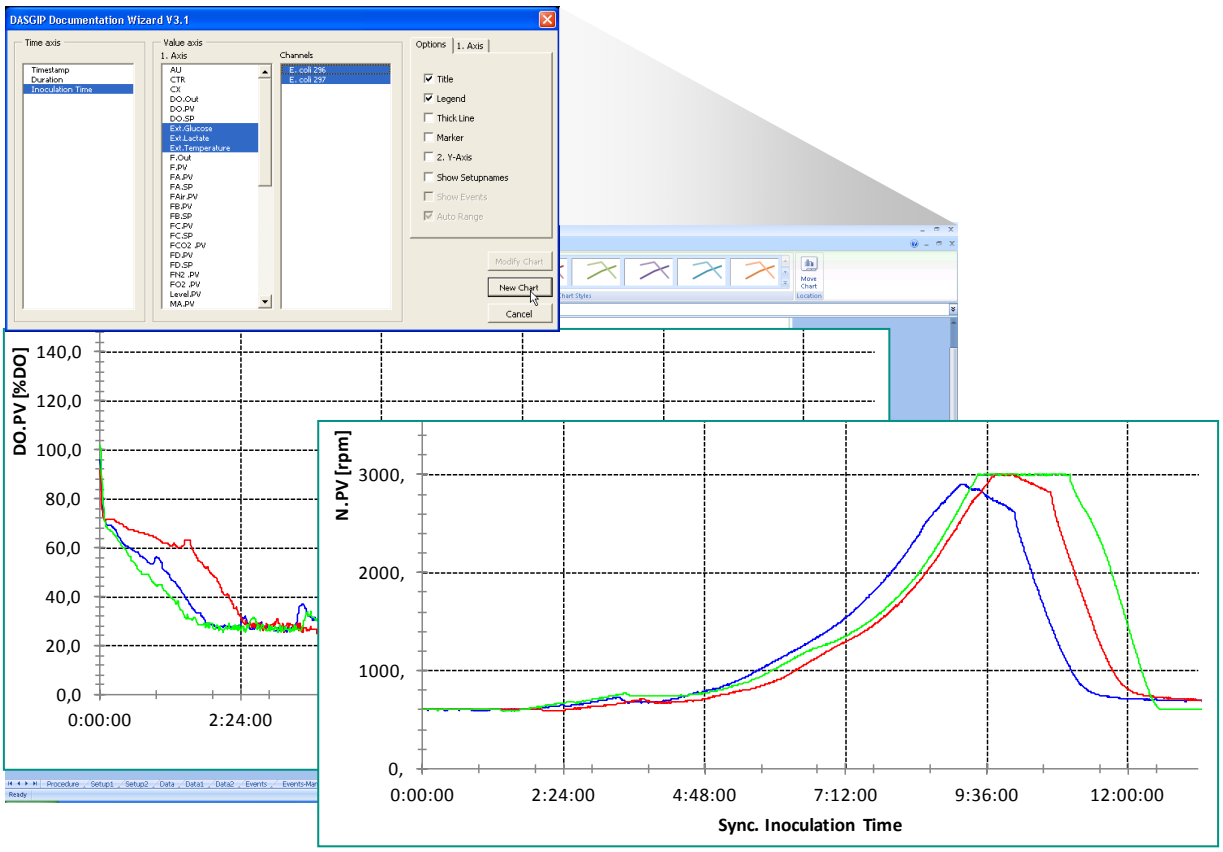
Workflow	Setup	Unit	User	T [°C]	pH [pH]	Gluc.Conc [g	OD600
Contains	Equal	Equal	Equal	Equal	Equal	Equal	Equal
KK107_DASbox_DoE-1-4	KK107_DASbox_DoE-1	Unit 1	Manager	37	6,8	40	45,4
KK107_DASbox_DoE-1-4	KK107_DASbox_DoE-2	Unit 2	Manager	40	7,2	60	46,7
KK107_DASbox_DoE-1-4	KK107_DASbox_DoE-3	Unit 3	Manager	34	7,2	60	65,8
KK107_DASbox_DoE-1-4	KK107_DASbox_DoE-4	Unit 4	Manager	40	7,2	20	23,6
KK107_DASbox_DoE-5-8	KK107_DASbox_DoE-5	Unit 1	Manager	40	6,4	60	67,4
KK107_DASbox_DoE-5-8	KK107_DASbox_DoE-6	Unit 2	Manager	37	6,8	40	47,6
KK107_DASbox_DoE-5-8	KK107_DASbox_DoE-7	Unit 3	Manager	34	7,2	20	25,6
KK107_DASbox_DoE-5-8	KK107_DASbox_DoE-8	Unit 4	Manager	40	6,4	20	26,1
KK107_DASbox_DoE-9-11	KK107_DASbox_DoE-9	Unit 1	Manager	34	6,4	60	56,8
KK107_DASbox_DoE-9-11	KK107_DASbox_DoE-10	Unit 2	Manager	34	6,4	20	33,6
KK107_DASbox_DoE-9-11	KK107_DASbox_DoE-11	Unit 3	Manager	37	6,8	40	50,9

- Export Report exports the entire process data, including recipes, process data history and events as a .csv file. This can be opened for further processing, e.g., for diagram creation in the *DASGIP Documentation Wizard*.

Reset View resets all query settings.

3.6. DASGIP Documentation Wizard

Evaluating and graphically displaying exported process data is particularly easy and convenient using the Microsoft Excel® add-in *DASGIP Documentation Wizard*. Both current and historical process data can be used and compared for the automatic creation of cultivation reports. Even individual ranges of process values, such as pH or DO, can be automatically grouped from different process datasets and displayed together graphically. It is recommended to select the process duration or directly the inoculation time as basis to ensure that the data series are automatically synchronized.



The synchronized process flows significantly facilitate plausibility checks.

All diagrams generated in the *DASGIP Documentation Wizard* can be formatted and edited with the standard Microsoft Excel® options.

More information can be found in the DASGIP Documentation Wizard Manual.

4. Application Example

Design of Experiments (DoE) is a method for the planning and statistical evaluation of experiments/processes. The goal of DoE is to learn as much as possible about the relationships between influencing variables (Input) and results (Output) with as little effort as possible.

DASware design, especially in combination with the *DASGIP Information Manager*, supports and synchronizes process management through the seamless integration of DoE.
The experiment design, which has been either created in the DoE Builder or imported from external DoE software tools, can be easily integrated and implemented in *DASGIP Control*.

DoE software tools can provide the experiment design that can be easily integrated and implemented in *DASGIP Control*.

This chapter outlines the implementation of a DoE strategy with and without DoE software tools (JMP) as an example.

4.1. Full Factorial DoE with Three Factors and Two Levels

A full factorial experiment setup consists of two or more factors with discrete values that are determined in two or more levels. In contrast to a fractional factorial setup, all factor combinations are carried out. This approach allows the observation or calculation of the effects of individual factors on the response as well as the interactions between the factors.

In the following, the cultivation of *Escherichia coli* is considered with three variable factors in two levels for the determination of optimal growth conditions. The response used is the bacteria biomass which is determined based on the determination of the optical density (OD_{600}). All possible combinations are carried out on *DASbox* System with three blocks.

Please keep in mind that this simple example is intended to teach - the outcome is evident.

Center Point Analysis

To obtain sufficient information about the accuracy of the process results, the use of repetitions is recommended in literature. However, this significantly increases the work involved. This is generally not feasible in industrial practice.

The selected experiment setup includes two levels per factor, so that a possible non-linear relationship (curvature) between the factors and the response cannot be eliminated. A center point determination will help to verify this. If the center point deviation is significant, then at least one factor has a non-linear influence.

It is important that the center point experiments are carried out together with the other experiments and not carried out later. If the experiment is divided into several blocks, a center point experiment should be implemented in each block. This is the only way of distinguishing between a deviation from the linearity and a change over time.

The threefold determination of the center point provides reliable information on the accuracy of the data at an economically justifiable cost.

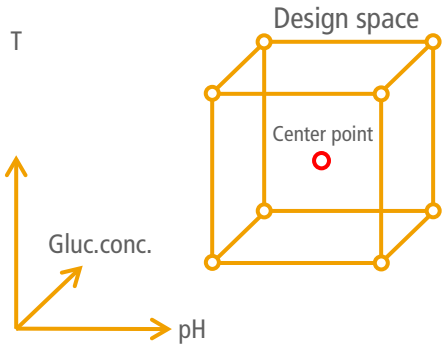
In addition, the influence of inoculation cultures (differences between reactor blocks) can be determined.

The selected settings are summarized below:

- Three factors with two levels and center points

Factors		Levels		
No.	Name	- 1	1	Center point
1	pH	6.8	7.2	7
2	T [° C]	34	37	35.5
3	Glucose concentration [g/L]	20	60	40

The corresponding three-dimensional experimental space (design space) with center point looks as follows:



- Response

Response	
No.	Name
R	Biomass (OD_{600})

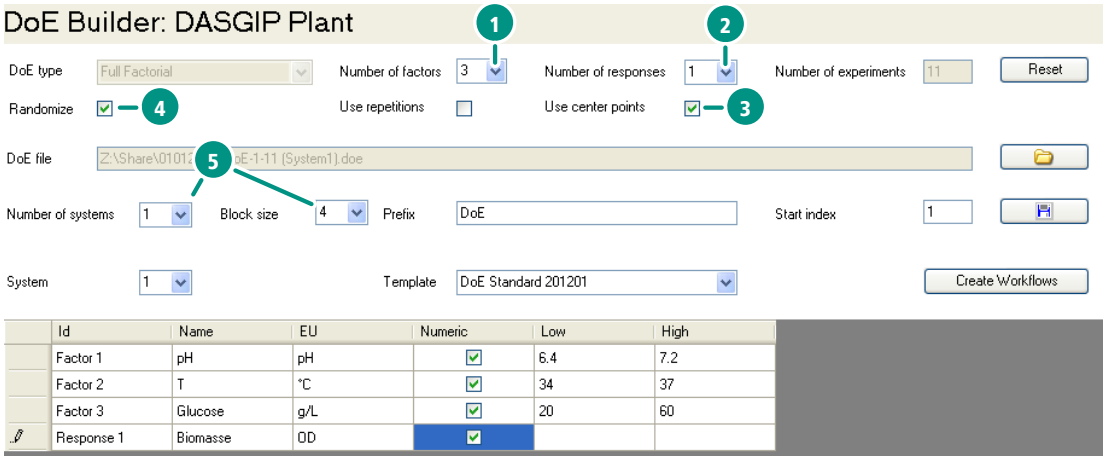
- Randomization

  The reactor positions are assigned to the three blocks. The assignment is random within the blocks to prevent a trend or any undetected fluctuation (e.g., sensor signal) from distorting the estimation of the effects of the factors.

4.1.1. Implementation without External DoE Software Tools

DoE Builder

The DoE Builder allows the desired entries to be entered directly:



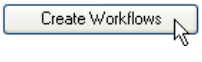
1. Select the number of factors: 3
2. Select the number of responses: 1
3. Select Use center points
4. Select Randomize
5. Select Number of systems 1 and Block size 4.

2^3 = 8 runs are needed. In addition, a center point is determined for each block so that a total of 11 runs are necessary. They are randomly distributed to system on an automatic basis and summarized in table form as follows:

Id	System	Workflow	Setup	Reactor	Block	Pattern	pH [pH]	T [°C]	Glucose [g/L]	Biomasse [OD]
4	1	DoE-1-4	DoE-1	1	1	++-	7.2	37	20	
1	1	DoE-1-4	DoE-2	2	1	---	6.4	34	20	
8	1	DoE-1-4	DoE-3	3	1	+++	7.2	37	60	
11	1	DoE-1-4	DoE-4	4	1	000	6.8	35.5	40	
10	1	DoE-5-8	DoE-5	1	2	000	6.8	35.5	40	
5	1	DoE-5-8	DoE-6	2	2	--+	6.4	34	60	
3	1	DoE-5-8	DoE-7	3	2	-+-	6.4	37	20	
6	1	DoE-5-8	DoE-8	4	2	+-+	7.2	34	60	
2	1	DoE-9-11	DoE-9	1	3	+--	7.2	34	20	
9	1	DoE-9-11	DoE-10	2	3	000	6.8	35.5	40	
7	1	DoE-9-11	DoE-11	3	3	-++	6.4	37	60	

The entries in the DoE Builder can be saved using the Save button in order to transfer them if required, e.g., to an additional *DASbox* PC.

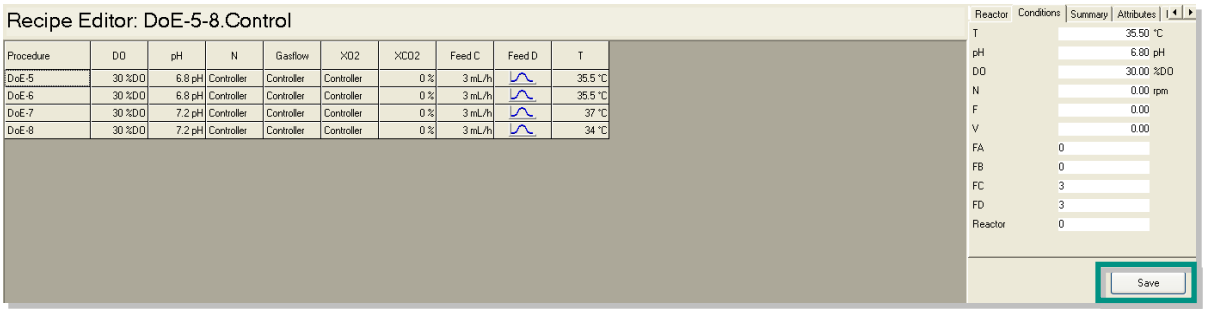
After the suitable workflow has been selected as a template, all entries are transferred with Create Workflow to the Recipe Editor.



Recipe Editor

In the Recipe Editor, the entries can be adjusted or supplemented before the process is implemented.

The second run (block) is shown as an example:



All entries are saved using the Save button and the process can be started using the Run button.

Process Monitoring and Control

During a process any manual operation in the Reactor View is possible. However we recommend avoiding any manual adjustment since they affect the DoE result potentially.
After all processes are completed, the process data is saved in *DASGIP Control* and can be used for further analysis.

DASGIP Information Manager

The *DASGIP Information Manager* is a helpful tool for performing plausibility checks and for complementing data. Process data can be grouped together with historical data and exported (see also 3.5).

Data Analysis

The exported process data can be generated by using the Export Table function and analyzed in Microsoft Excel®.

- Most favorable factor combinations

Temp A	pH B	Gluc.-conc. C	OD600 (t1)
-	-	-	33,6
+	-	-	26,1
-	+	-	25,6
+	+	-	23,6
-	-	+	56,8
+	-	+	67,4
-	+	+	65,8
+	+	+	46,7
center	center	center	48,0

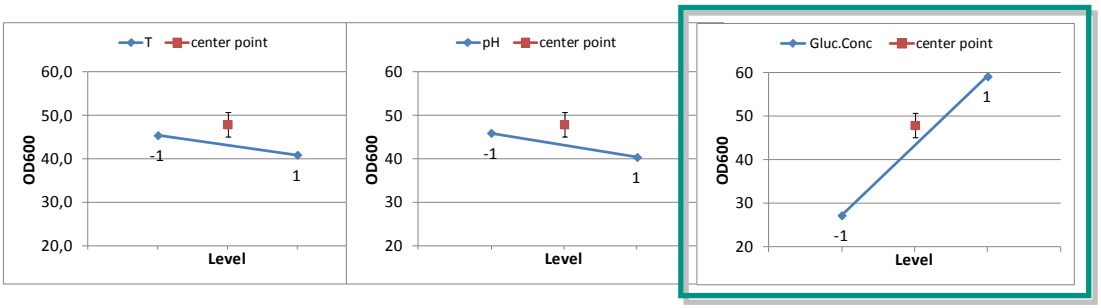
The most favorable factor combination is determined by the highest biomass concentration.

- Main effects

The main effects are analyzed by calculating the average values for the high (+) and the low (-) factor level. The difference of the average values is the effect in question.

Temp A	pH B	Gluc.-conc. C	
41,0	40,4	59,2	**Average Level "-"**
48,0	48,0	48,0	**Average Center Points**
45,5	46,0	27,2	**Average Level "+"**
-4,5	-5,6	32,0	**Effect**

It is obvious that the factor glucose concentration has the largest effect on the response biomass. This is represented graphically with 1 and -1 for the two levels below.

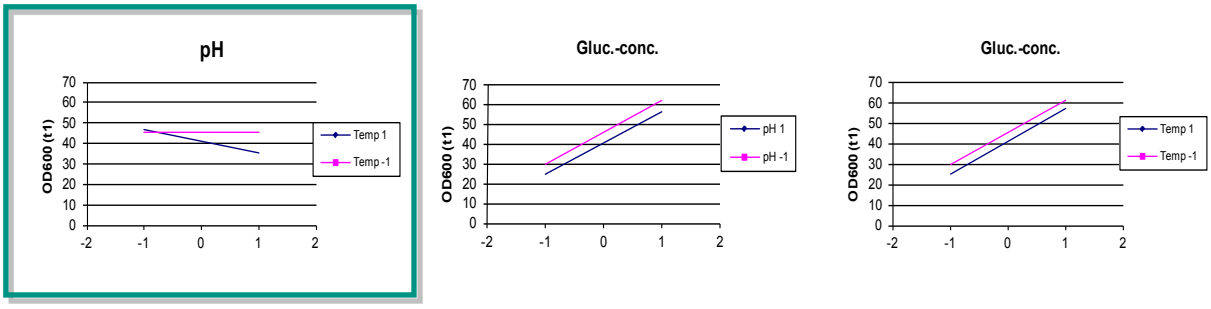


In addition, the determined center points provide information about a possible deviation from linearity. The 3-fold implementation of the center point determination used in this example provides an indication of the chance variation.

- Interactions

The figures below show the average values of both levels of a factor depending on the setting of an additional factor.

An indicator of an interaction is the degree of non-parallelism between the corresponding straight lines. The greater the degree of parallelism between the straight lines, the lower the interactions.

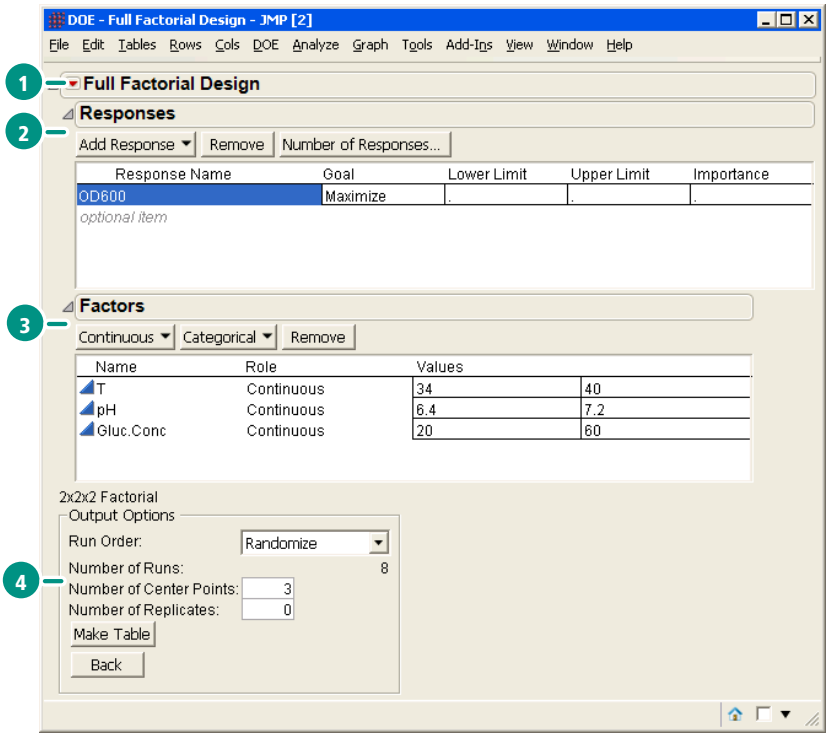


The presented results indicate an interaction between the factors pH and temperature, because the straight lines intersect. In the subsequent stage of process optimization, these two factors should be considered together.

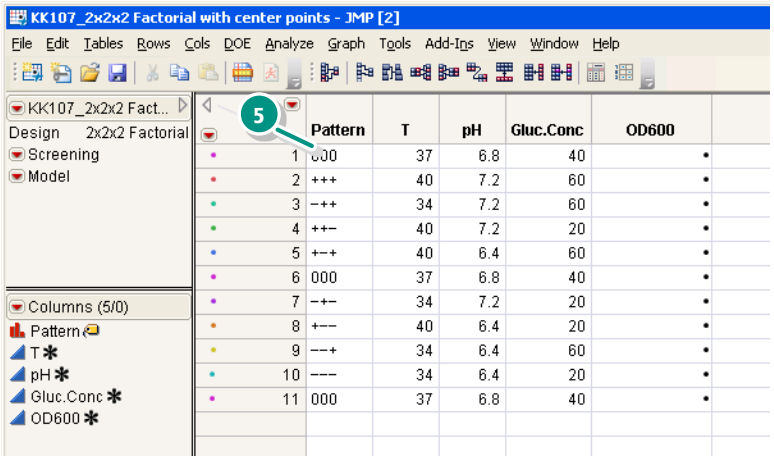
4.1.2. Use of External DoE Tools (JMP)

JMP is a standard software tool for the statistical planning of experiments, such as DoE. The following screenshots briefly outline the procedure for an experiment setup using JMP.

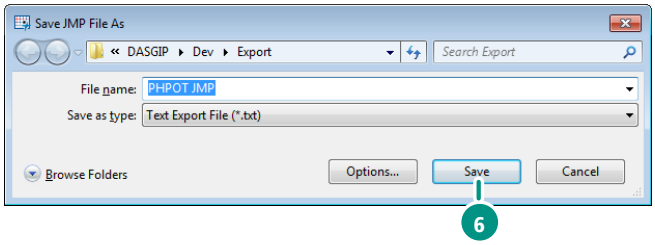
1. Define design
2. Define responses
3. Define factors
4. Define center points



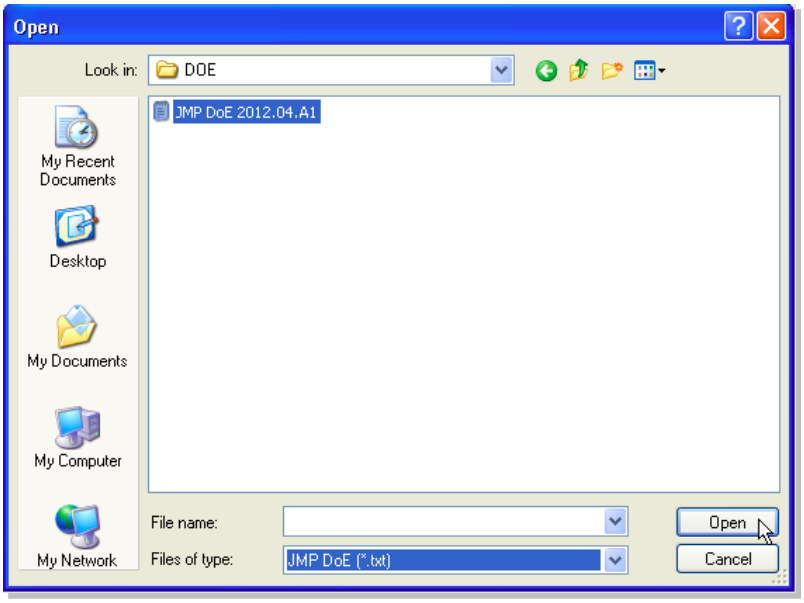
5. Generate a table with all entries



Save the table as a Text Export File (*.txt) type.

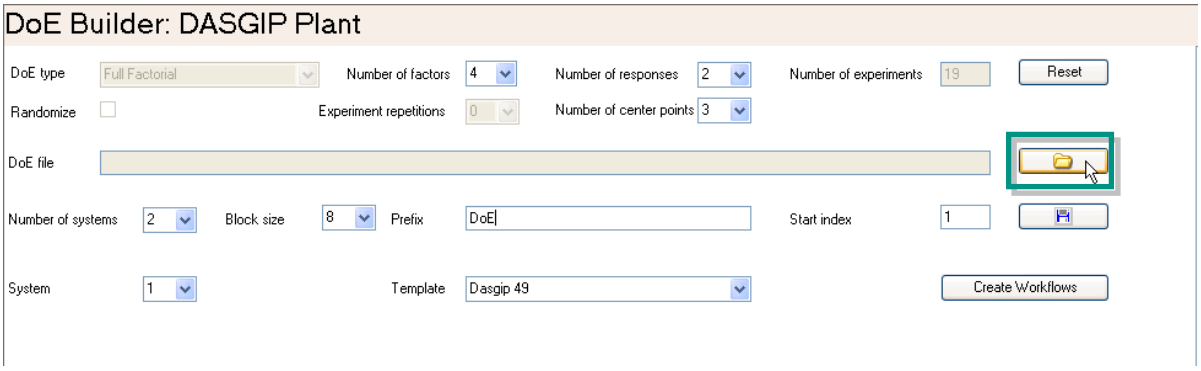


This table generated and saved in JMP can now be easily loaded to the DoE Builder (4.1.1) of *DASGIP Control* so that all entries are automatically adopted.



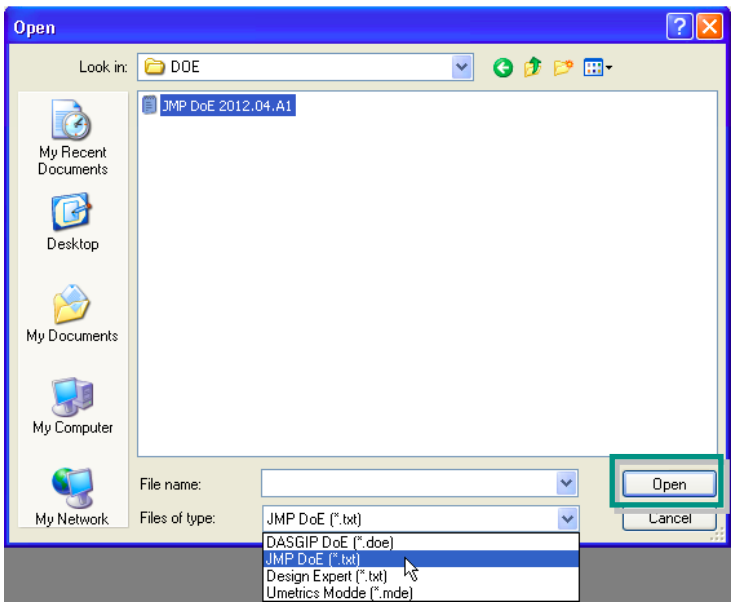
Loading External DoE Data to the DoE Builder

DASware design offers the possibility to seamlessly integrate DoE data from different DoE software tools, such as JMP, in *DASGIP Control*.



Click on the  button to open the following dialog.

Select a file and click on the Open button to open the file choosing the appropriate File type.



The DoE data is then loaded into the DoE Builder and can still be adjusted if necessary.

Id	System	Workflow	Setup	Reactor	Block	Pattern	pH [pH]	T [°C]	Glucose [g/L]	Biomasse [OD]
4	1	DoE-1-4	DoE-1	1	1	++-	7.2	37	20	
1	1	DoE-1-4	DoE-2	2	1	---	6.4	34	20	
8	1	DoE-1-4	DoE-3	3	1	+++	7.2	37	60	
11	1	DoE-1-4	DoE-4	4	1	000	6.8	35.5	40	
10	1	DoE-5-8	DoE-5	1	2	000	6.8	35.5	40	
5	1	DoE-5-8	DoE-6	2	2	--+	6.4	34	60	
3	1	DoE-5-8	DoE-7	3	2	-+-	6.4	37	20	
6	1	DoE-5-8	DoE-8	4	2	+-+	7.2	34	60	
2	1	DoE-9-11	DoE-9	1	3	+--	7.2	34	20	
9	1	DoE-9-11	DoE-10	2	3	000	6.8	35.5	40	
7	1	DoE-9-11	DoE-11	3	3	-++	6.4	37	60	

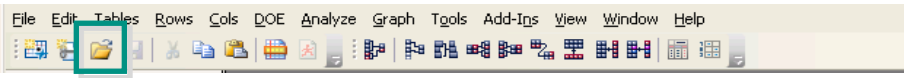
Process Monitoring and Control

The process is executed as described under 4.1.1.

After all processes are completed, the process data is saved in *DASGIP Control* and can be used for further analysis. Use the documentation wizard to select and export the factor and response table.

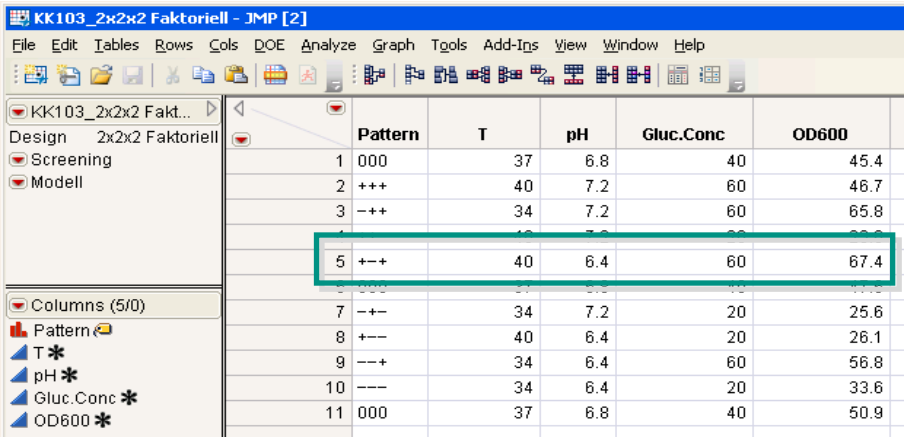
Data Analysis

The exported DoE text file can be opened using the Open button in JMP.



- Most favorable factor combinations

The most favorable factor combination is determined by the highest biomass concentration.

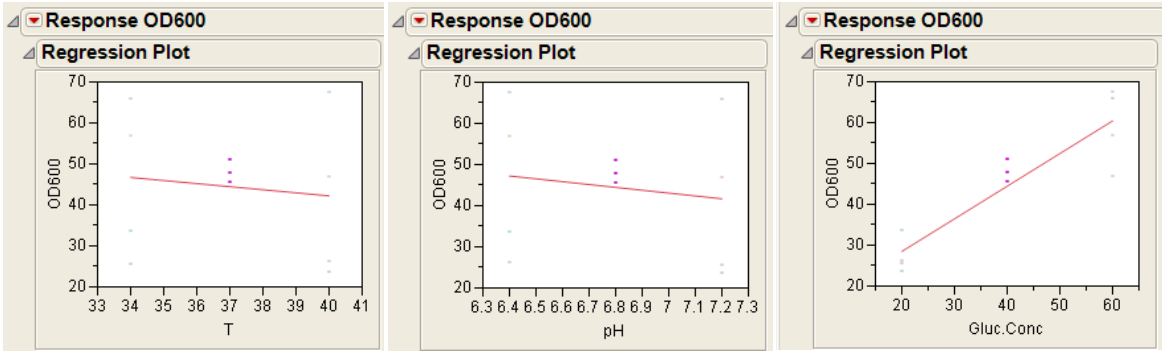


	Pattern	T	pH	Gluc.Conc	OD600
1	000	37	6.8	40	45.4
2	+++	40	7.2	60	46.7
3	−++	34	7.2	60	65.8
5	+−+	40	6.4	60	67.4
7	−+−	34	7.2	20	25.6
8	+−−	40	6.4	20	26.1
9	−−+	34	6.4	60	56.8
10	−−−	34	6.4	20	33.6
11	000	37	6.8	40	50.9

- Main effects

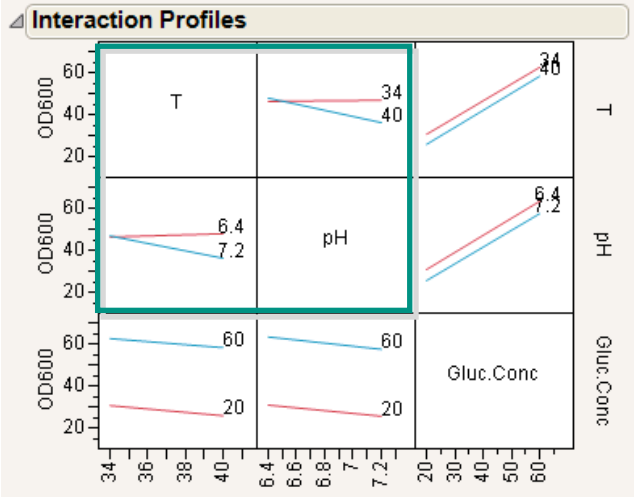
The main effects are analyzed at this point using a linear regression. The gradient is an indication of the effect of a factor.

In the following, it can be seen that the glucose concentration has the largest effect on the response biomass.



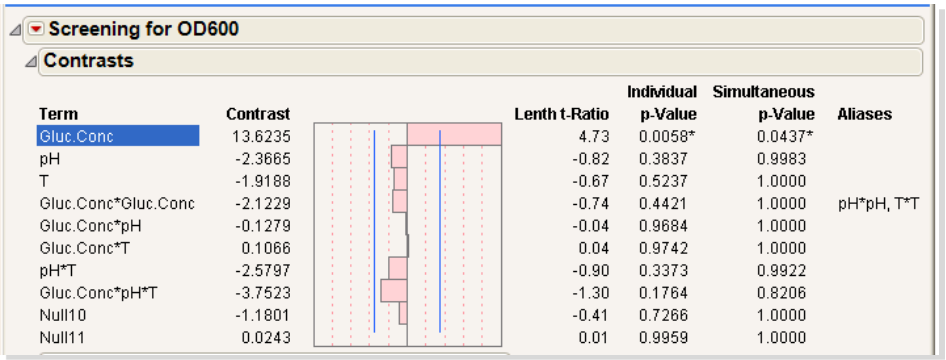
- Interactions

The average values of both levels of a factor are also shown here depending on the setting of another factor.



The results indicate an interaction between the factors pH and temperature. These should therefore be considered together in a subsequent process optimization stage.

The following shows an additional display to determine the significance of effects, both of individual factors as well as of factor-factor interactions.



Term	Contrast	Lenth t-Ratio	Individual p-Value	Simultaneous p-Value	Aliases
Gluc.Conc	13.6235	4.73	0.0058*	0.0437*	
pH	-2.3665	-0.82	0.3837	0.9983	
T	-1.9188	-0.67	0.5237	1.0000	
Gluc.Conc*Gluc.Conc	-2.1229	-0.74	0.4421	1.0000	pH*pH, T*T
Gluc.Conc*pH	-0.1279	-0.04	0.9684	1.0000	
Gluc.Conc*T	0.1066	0.04	0.9742	1.0000	
pH*T	-2.5797	-0.90	0.3373	0.9922	
Gluc.Conc*pH*T	-3.7523	-1.30	0.1764	0.8206	
Null10	-1.1801	-0.41	0.7266	1.0000	
Null11	0.0243	0.01	0.9959	1.0000	

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