Retsch®
Solutions in Milling & Sieving
part of VERDER scientific

Fast and reproducible method for cell disruption

Using the Mixer Mill MM 400 to disrupt up to 8 x 25 ml cell suspension simultaneously

AUTHOR

Dr. Tanja Butt

Product Management

Retsch®
Solutions in Milling & Sieving

Retsch GmbH
Retsch-Allee 1-5
42781 Haan, Germany

Phone: +49 (0)2104/2333-100
E-Mail: t.butt@retsch.com

www.retsch.com

Cell disruption of bacteria, yeast, filamentous fungi or microalgae is a standard procedure in basic biological research, applied biotechnology or medical research to get access to nucleic acids (DNA, RNA) or cell proteins. For the isolation of DNA or RNA usually less than 1 ml of cell material is needed. For the extraction of proteins, however, larger amounts of cell suspension are required. A very efficient method of cell disruption is the co called "bead beating" where cells in suspension are mechanically disrupted by glass beads in single-use reaction vials.

A very basic method is to hold the reaction vial over a Vortexer, thus dispersing cell suspension and glass beads in the vial which leads to breaking of the cell walls by shearing effects. This method is time-consuming and error-prone, particularly for high sample throughput or cell disruption times up to 10 minutes. The use of RETSCH's Mixer Mill MM 400 in combination with the Falcon tube adapter, which accepts up to 8 tubes, makes the process reproducible, fast and efficient. The effectiveness of the method is demonstrated in this application alert using the examples of cell disruption of yeast cells (*Saccharomyces cerevisiae*) and microalgae cells (*Thalassiosira pseudonana* and *Phaeodactylum tricornutum*).

Retsch
Solutions in Milling & Sieving

Cell disruption of yeast suspensions

in the Department for Systems Biochemistry at the Institute for Biochemistry and Pathology of Ruhr University Bochum, Germany

The Department for Systems Biochemistry of the Ruhr University carries out research on cellular components, the so-called peroxisomes. These are involved, for example, in the degradation of fatty acids in eukaryotic cells. Peroxisomes which are not fully functional can lead to severe metabolic disorders. To get a better understanding of the functioning of peroxisomes, the PhD student Anna Chan studies the transportation processes of cellular ingredients in and out of the peroxisomes using *Saccharomyces cerevisiae* as a model organism. For these investigations it is necessary to disrupt volumes of approximately 20 ml cell suspension to study the functionality of several cell proteins. In the past, cell disruption was performed by manual vortexing of 4 g yeast cells with 12 g disruption buffer and 16 g glass beads (0.5 mm to 0.75 mm in diameter) in 50 ml Falcon tubes. The procedure took 12 x 1 min with 1 min intermediate cooling on ice. Simultaneous disruption of more than two samples could only be achieved by having a number of people working on several vortexers in a row. Moreover, it was mandatory to exchange the samples among the operators to compensate for user-specific and hardware-specific differences. The use of the Mixer Mill MM 400 greatly simplifies work for Anna Chan, especially the adapter for simultaneous operation of up to eight 50 ml Falcon tubes. With this mill several samples of cell suspension are disrupted automatically with up to 30 Hz. For validation reasons, Miss Chan compared the total protein content derived after manual cell disruption on a Vortexer with that obtained with the automatic method using the MM 400. Another aspect to be considered was the temperature development in the cell suspension during disruption and the reproducibility of both methods (figures 1 and 2).

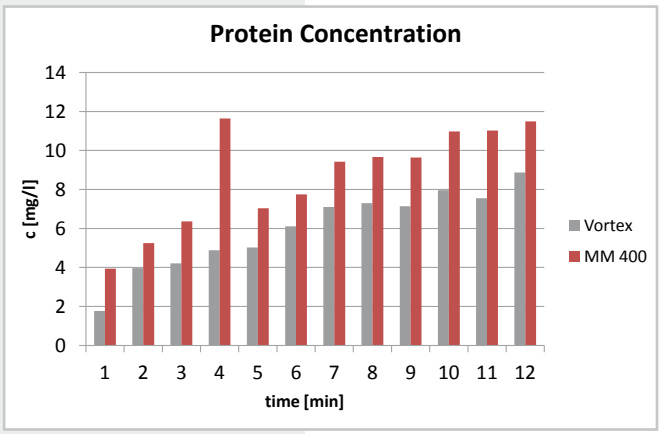

Fig. 1: Total protein concentration of the disrupted cell suspension (left) and temperature increase during cell disruption (right)

After only 6 to 7 minutes the same protein concentration was measured after cell disruption at 30 Hz in the MM 400 as after 12 minutes of manual cell disruption using a Vortexer. Moreover, with a temperature difference of approximately 8°C the increase was lower than during cell disruption with the Vortexer which amounted to 12 °C. The comparison also showed that the bead beating method using the Mixer Mill MM 400 provides better reproducibility with only 0.2-4% standard deviation compared to 0.9-9%.

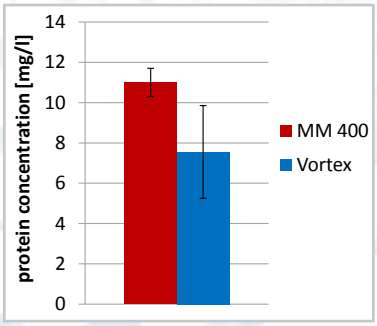

Fig. 2: Better reproducibility of total protein concentration after 11 minutes of cell disruption with MM 400 in comparison to manual vortexing (error bars as % deviation)

Retsch
Solutions in Milling & Sieving

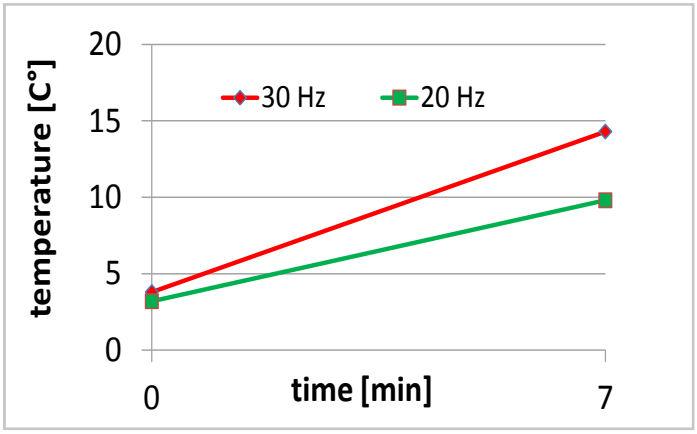

Fig. 3: Increase of temperature during 7 min of cell disruption at 20 Hz or 30 Hz, no intermediate cooling on ice

Cell disruption results were slightly improved by using glass beads sized 0.75 mm to 1.00 mm instead of 0.5 mm to 0.75 mm. For the extraction of proteins it was beneficial to decrease the speed of cell disruption to 20 Hz, resulting in less foaming and a slight increase in total protein content. Moreover, the temperature increase was less at 20 Hz compared to 30 Hz (figure 3). Miss Chan could show in activity assays that the extracted proteins were functional.

Cell disruption of microalgae

at the Institute for Molecular Biosciences, Goethe University Frankfurt, Germany

Dr. Frederik Barka, researcher at the Institute for Molecular Biosciences at the Goethe University Frankfurt, investigates the composition and function of proteins involved in photosynthesis of microalgae (Diatoms). Cell disruption posed a problem since the cells tend to withstand the shearing effects produced by the glass beads. Cell disruption of 2 ml volumes using glass beads in combination with mills of other manufacturers was not successful so that the cells were disrupted via French Press. In contrast to previous experiences, cells are now successfully disrupted by glass beads using the Mixer Mill MM 400 in combination with the Falcon tube adapter. 300 ml cell suspension of the organism *Thalassiosira pseudonana* were centrifuged, re-suspended in 20 ml disruption buffer and filled in a 50 ml Falcon tube. 40 ml glass beads (90 µm to 150 µm and 300 µm to 400 µm, ratio 1:1) were added and cell disruption was performed for 20 sec at 20 Hz. Complete cell disruption was visible through the microscope (figure 4).

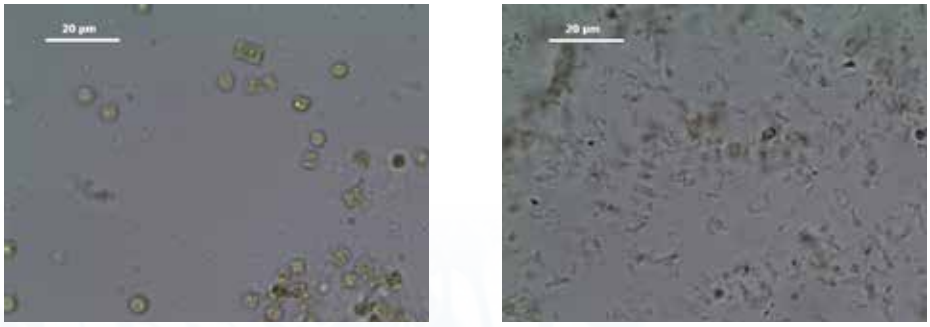

Fig. 4: Cell of the diatom *Thalassiosira pseudonana* before (left) and after disruption (right) using the Mixer Mill MM 400 in combination with the Falcon tube adapter

The MM 400 even achieved full cell disruption of the algae *Phaeodactylum tricornutum* which easily withstands shearing effects thanks to a missing silicate cell wall. 200 ml cell culture were centrifuged and re-suspended in 20 ml disruption buffer and transferred to a 50 ml Falcon tube. 40 ml glass beads (90 µm to 150 µm and 300 µm to 400 µm, ratio 1:1) were added and cell disruption was performed within 3 x 60 sec at 30 Hz. After a total of 3 minutes no intact cells were visible through the microscope (figure 5).

Retsch
Solutions in Milling & Sieving

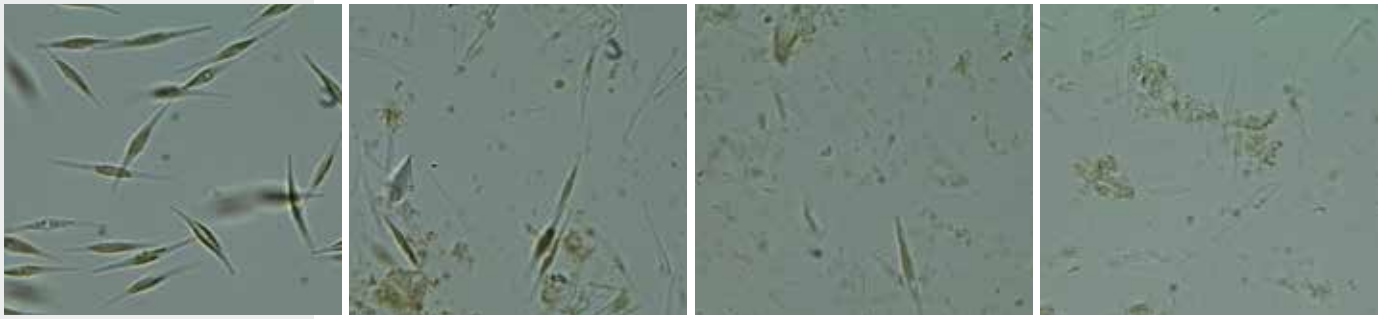

Fig. 5: Cells of *Phaeodactylum tricornutum* before (left) and after cell disruption (right) with the Mixer Mill MM 400 in combination with the Falcon tube adapter, 3 x 60 sec at 30 Hz

Mixer Mill MM 400 - a true multi-purpose mill in the lab

RETSCH's Mixer Mill MM 400 is very versatile in use thanks to a wide range of accessories (figure 6). It is ideally suited for cell disruption in single-use vials, such as 50 ml Falcon tubes or smaller reaction vials like Eppendorf tubes. The MM 400 is used for the disruption or grinding of a huge variety of hard, semi-hard, brittle and soft, elastic or fibrous materials, including plants, pine needles, feathers, bones, cell tissue, pharmaceutical products, wood, minerals or chemicals, making it a true multi-purpose mill when it comes to sample preparation. It achieves grind sizes down to 5 µm.

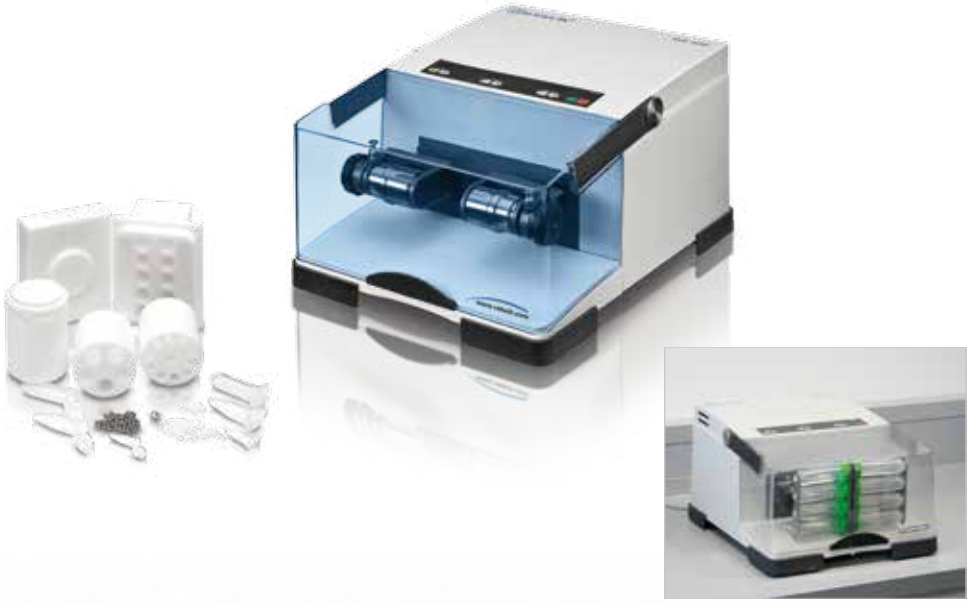

Fig. 6: Mixer Mill MM 400 with adapters for Eppendorf tubes (left) and adapter for 8 x 50 ml Falcon tubes (right)

Conclusion

Cell disruption by bead beating is a very effective and reliable method greatly superior to the manual procedure involving a Vortexer. RETSCH's Mixer Mill MM 400 accepts up to 8 Falcon tubes (50 ml) or up to 20 single-use tubes (2 ml) thus allowing for high throughput and ensuring a reproducible, fast and efficient cell disruption process with very good results.

www.retsch.com/mm400