Appl Microbiol Biotechnol (2008) 81:431–440
DOI 10.1007/s00253-008-1660-6

BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Influence of processing parameters on disintegration of *Chlorella* cells in various types of homogenizers

J. Doucha · K. Lívanský

Received: 4 June 2008 / Revised: 6 August 2008 / Accepted: 6 August 2008 / Published online: 29 August 2008

Abstract The following bead mills used for disruption of the microalga *Chlorella* cells were tested: (1) Dyno-Mill ECM-Pilot, grinding chamber volume 1.5 L; KDL-Pilot A, chamber volume 1.4 L; KD 20 S, chamber volume 18.3 L; KD 25 S, chamber volume 26 L of Willy A. Bachofen, Basel, Switzerland, (2) LabStar LS 1, chamber volume 0.6 L of Netzsch, Selb, Germany, (3) MS 18, chamber volume 1.1 L of FrymaKoruma, Neuenburg, Germany. Amount of disrupted cells decreased with increasing *Chlorella* suspension feed rate and increased up to about 85% of the beads volume in the grinding chamber of the homogenizers. It also increased with agitator speed and number of passes of the algae suspension through the chamber. The optimum beads diameter was 0.3–0.5 mm in the homogenizers Dyno-Mill and LabStar LS 1 and 0.5–0.7 mm in the homogenizer MS 18. While the degree of the cell disruption decreased with increasing cell density in Dyno-Mill and LabStar, the cell disruption in the MS 18 increased. Depending on processing parameters, more than 90% of algae cells were disrupted by passing through the bead mills and bacteria count in algae suspension was reduced to about two orders.

Keywords Processing parameters · Disintegration efficiency · *Chlorella* · Bacteria reduction

Introduction

Cell disruption is often necessary for recovering intracellular products from microalgae (Mendes-Pinto et al. 2001, Molina Grima et al. 2003). The rigid cellulosic cell wall, one of the characteristics of unicellular microalgae such as *Chlorella*, causes a low utilization of the algal cell content by organism of a recipient. The digestibility of non-ruptured *Chlorella*-cell content ranges mostly from 15–25% (Becker 1994). The disruption of the cells increases this value (estimated as protein digestibility after 4 h of in vitro pepsin treatment) up to 80% of that of casein. In order to open the cell, various ways were tested, including, e.g., freezing, alkalic and organic solvents, osmotic shocks, sonication, high pressure homogenization, and bead milling (Wimpenny 1967; Chisti and Moo-young 1986, Molina Grima et al. 2004). Industrially relevant are horizontal bead mills (Kula and Schütte 1987; Middelberg 1995) originally designed for the homogenization and size reduction of different commercial products such as milk and paint. The principle of bead mills is based on rapid stirring of a thickened suspension of microorganisms in the presence of beads. The basic setup of a bead mill is a jacketed grinding chamber with a rotating shaft through its centre. The shaft is fitted with discs that impact kinetic energy to small beads in the chamber, forcing them to collide with each other. The beads are retained in a grinding chamber by a sieve or an axial slot smaller than the bead size. The beads are accelerated in a radial direction, forming stream layers of different velocity and creating high shear forces. An external pump feeds the suspension into the grinding chamber. For research and development, laboratory mills with working volume up to 5 l are offered for continuous and batch operations. Results obtained can serve to find suitable operating conditions for larger production machines, which, however, require some experimental verification (Schütte and Kula 1990).

Disintegration of baker's yeast and some bacteria using bead mill was pioneered many years ago in former Czechoslovakia (Novotný 1964; Řeháček et al. 1969; Řeháček 1971). However, very limited information dealing with the disintegration of algal cells can be found in literature. In spite of it, more and more big Asian *Chlorella* producers have been

J. Doucha (✉) · K. Lívanský
Division of Autotrophic Microorganisms, Institute of Microbiology, Academy of Sciences of the Czech Republic, 379 81 Třeboň, Czech Republic
e-mail: doucha@alga.cz

aware of the importance of this step and include mechanical disintegration of algal cells to the downstream processing line. In our laboratory, the technology of cell disintegration by means of a bead mill, originally used for homogenization of inorganic pigments, was developed and included into the downstream processing of microalgae many years ago (Hedenskog et al. 1969, Doucha 1998).

The extent of cell disruption depends on the residence-time distribution (RTD), shear forces, type of microorganism, cells concentration etc. Again, the RTD is influenced by feed rate, chamber volume, agitator construction and speed, beads volume, etc. Mölls and Hörnle (1972) identified about 40 parameters influencing cell disruption. The high number of parameters should not be considered a disadvantage since it opens up possibilities for optimizing performance for different products. The most important processing parameters for disintegration, which can be controlled directly, are: feed rate of the suspension, agitator speed, cell density, bead diameter, bead density, bead filling (% of the grinding chamber volume), geometry of the grinding chamber, and design of the stirrer (Kula and Schütte 1987, Engler 1993, Hatti-Kaul and Mattiasson 2003). Because of a larger number of factors which may affect disruption of microbial cells, no comprehensive theory of this process is available in literature.

The aim of this work was to test the influence of the following processing parameters: flow (feed) rate through the disintegration (grinding) chamber, agitator speed, cell density, size of beads, beads filling, and number of passes of the suspension through the chamber on the degree of disintegration of the cells in thickened (50–150 g dry weight l^{-1}) *Chlorella* suspension. Algae cultures grown in outdoor bioreactors contain accompanying bacterial microflora. That is why the influence of the above-mentioned parameters on reduction of bacteria caused by disintegration was also investigated.

Materials and methods

The homogenizers (bead mills) of the following European manufacturers were used: Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland—ECM-Pilot, KDL-Pilot A, KD 20 S, KD 25 S; Netzsch, Selb, Germany—LabStar LS 1; FrymaKoruma, Neuenburg, Germany—MS 18. Some characteristics of the homogenizers are given in Table 1.

The homogenizer Dyno-Mill ECM-Pilot operated with a recirculation of thickened algae suspension, the other homogenizers investigated in this work operated with continuous feeding of the suspension into the disintegration chamber.

The Dyno-Mill and LabStar LS 1 are bead mills with a horizontal cylindrical grinding chamber. The product is pumped into the chamber, moved through it and exposed for a certain time to the stress of the moving grinding beads. Specially designed agitator discs, mounted symmetrically on a shaft, transfer the energy required for dispersion and wet grinding to the spherical grinding beads. Most of the energy introduced by the agitator discs is converted to heat, which is carried off by means of a cooling liquid, which circulates in a cooling jacket of the grinding chamber. Before the product leaves the chamber, the beads are separated from the product by means of a separation system. Number of agitator discs differs according to the homogenizer type (e.g. KDL-Pilot A—five discs, KD 20 S—six discs, KD 25 S—nine discs).

The homogenizer FrymaKoruma MS 18 is used for fine grinding of suspensions and high viscous products. The product is fed to the grinding chamber by an external pump. A milling zone is created in the gap between a conical working vessel—the stator—and a conical rotor. The movement of the rotor creates radial movement of the grinding media (beads). Momentum amplifies the outward motion, so that the product shear force increases steadily during the milling operation. The beads are automatically re-introduced into the product flow as it enters the grinding chamber, so that continuous circulation of the media in the chamber is achieved. The design of the grinding chamber ensures that all product particles are subjected to the same grinding efficiency. The beads and product are separated at the end of the chamber by a sieve. The heat is carried off by means of a cooling liquid circulating in a cooling jacket of the grinding chamber. The peripheral speed of the rotor, the grinding gap, the material, and the diameter, as well as the volume of the grinding beads and the throughput of the suspension, influence the grinding efficiency.

Table 1 Characteristics of the homogenizers

Mill type	Grinding chamber volume (l)	Installed power (kW)	Capacity[a] (kg h^{-1})
ECM-Pilot, Dyno-Mill	1.5	7.5	10–200
KDL-Pilot A, Dyno-Mill	1.4	3.3	8–40
KD 20 S, Dyno-Mill	18.3	25	70–500
KD 25 S, Dyno-Mill	26	25	100–700
LabStar LS 1, Netzsch	0.6	3	4–25
MS 18, FrymaKoruma	1.1	11	20–80

[a] Depending on product characteristics

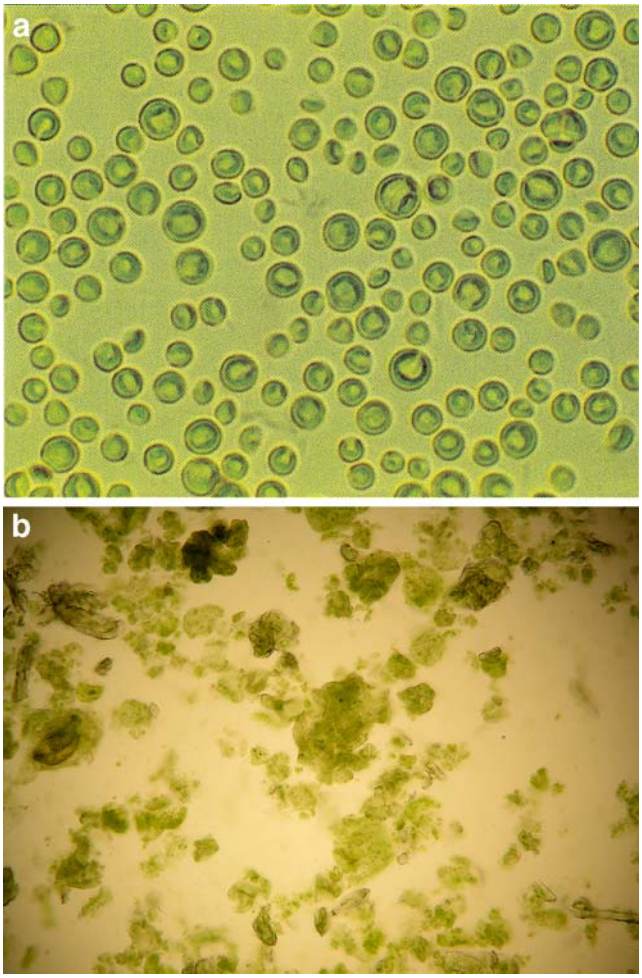

Fig. 1 Suspension of *Chlorella* cells before (**a**) and after disintegration (**b**) (DynoMill KDL-Pilot A, biomass density 107 g l^{-1}, glass beads 0.42–0.58 mm, feed rate 5 kg h^{-1}, discs speed 14 m s^{-1}; degree of disintegration 99%)

Culture of chlorococcal unicellar alga *Chlorella*, strain *P12* (from the collection in our laboratory) grown outdoors in solar thin-layer photobioreactor (Doucha and Lívanský 1995, 1999, 2006) was thickened with a continuous self-desludging plate centrifuge and cooled to 3–4°C before the cell disruption. Typical *Chlorella* cells volume distribution before disintegration is given in Fig. 2 (curve a). Mean cell volume was 41 μm^3, corresponding to a mean cell diameter about 4.3 μm.

Degree of disintegration of *Chlorella* cells was estimated by counting numbers of non-ruptured cells in a Bürker chamber before (cell count n_0) and after (cell count n) disintegration, as: Des (%)=$100\times(n_0-n)/n_0$.

Algae dry weight was estimated after centrifugation (10,000×*g*, 3 min) of 2 ml of algal suspension in an Eppendorf tube, then drying at 105°C for at least 8 h. The determination was made in triplicate for each sample.

The count of viable bacteria in algae suspension (before and after the disintegration) was determined after the appropriate dilution of the suspension and cultivation on Petri dishes containing sterilized agar enriched with 0.09 M NaCl solution. After incubation period (48 h at 33°C), the number of bacteria colonies was estimated. Reduction of viable bacteria count in algae suspension passed through the disintegration chamber was estimated as: Red (%)=$100\times(n_0-n)/n_0$, where n_0 is the number of viable bacteria in 1 ml volume of non-disintegrated and n is the number of viable bacteria in 1 ml volume of disintegrated suspension of algae cells.

Results

A view of the suspension of *Chlorella* cells before and after disintegration is given in Fig. 1. It is apparent from Tables 3, 4, and 5 that at a suitable combination of processing parameters, almost all algae cells can be disrupted by a single pass of suspension through the homogenizers.

Typical particle volume distributions in suspension of non-disintegrated and disintegrated algae cells are given in Fig. 2.

Homogenizers Dyno-Mill

Influence of disintegration time t (min) and beads diameter BD (mm) on the degree of cells disintegration Des (%) in the homogenizer Dyno-Mill ECM-Pilot was investigated—Table 2. The data were correlated as:

$$\mathrm{Des}(\%) = a_0 \mathrm{BD}^{n_1} t^{n_2} \quad (1)$$

with a_0=27.86; n_1=0.154; n_2=0.307; correlation coefficient r=0.997.

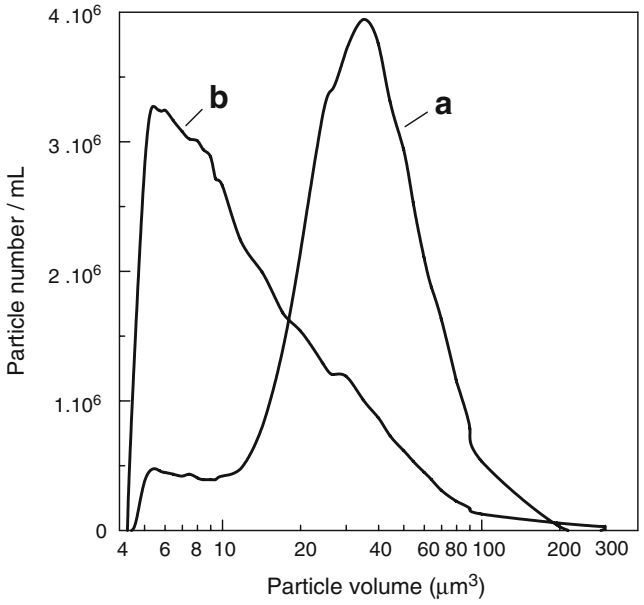

Fig. 2 Particle volume distribution in 4,000× diluted suspension of non-disintegrated (curve *a*) and disintegrated (curve *b*) algal cells. Parameters of disintegration—see Fig. 1

Table 2 Influence of disintegration time and beads diameter on the degree of cells disintegration in the homogenizer Dyno-Mill ECM-Pilot

Trial no	Algae dry weight g l^{-1}	Feed rate kg h^{-1}	Beads filling (% of chamber volume)	Speed of agitator discs m s^{-1}	Disintegration time min	Degree of cells disintegration %
Glass beads 0.25–0.5 mm						
1	158	62	70	10	30	67
2					60	85
3					90	95
ZrO2 beads 0.5 mm						
1	158	62	70	10	30	71
2					60	89
3					90	98.5

Assuming that the decrease of the algae cell number in the course of disintegration in a homogenizer is of first-order kinetics, i.e.:

$$-dn/dt = k_1 n \tag{2}$$

by integration of this equation for the initial conditions: $t=0$, $n=n_0$, we obtained for the number of non-disintegrated cells with a mean residence time T in a grinding chamber:

$$n = n_0 \exp(-k_1 T) \tag{3}$$

where k_1 is a rate constant. From the definition of the degree of disintegration and Eq. 3, it follows:

$$\mathrm{Des}(\%) = 100[1 - \exp(-k_1 t)] \tag{4}$$

We attempted to verify this relationship from the data (Table 2) for the Dyno-Mill ECM-Pilot where the algae suspension was re-circulated through the disintegration chamber. We found: $k_1=3.488\times10^{-2}$ min^{-1}, $r=0.985$, for the beads diameter 0.25–0.5 mm; $k_1=4.023\times10^{-2}$ min^{-1}, $r=0.992$, for the beads diameter 0.5 mm. It is seen that the r

Table 3 Degree of algae cells disintegration and reduction of bacteria count in the homogenizers Dyno-Mill

Trial no	Algae dry weight (g l^{-1})	Feed rate kg (h^{-1})	Beads filling (% of chamber volume)	Speed of agitator discs (m s^{-1})	Degree of cells disintegration (%)	Reduction of bacteria count (%)
a) Dyno-Mill KDL-Pilot A						
Glass beads 0.15–0.25 mm:						
1	112	10.2	82	10	55	67.6
2		15		14	70	73.1
Glass beads 0.2–0.3 mm:						
3	107	20	82	14	92.5	78.7
Glass beads 0.42–0.58 mm:						
4	107	3	82	10	99	85.7
5		5			96.5	83.8
6		8			96.5	79.8
7		3		14	99.9	90.2
8		5			99	87.9
9		15			97	84.2
10		40			90	76.2
11	112	10.2		10	80	75.3
12				14	92	78
ZrO2 beads 0.3 mm:						
13	102.5	3	85	14	92.3	84.3
14		10			83.2	80.9
15		15			77.9	76.7
b) Dyno-Mill KD 20 S						
ZrO2 beads 0.3–0.4 mm:						
1	124.2	120	75	13	58.1	69.4
2		38	75		75.2	72.1
3		35	85		82.8	76.3
ZrO2 beads 0.6–0.8 mm:						
4	124.2	35	85	13	85.2	81.2
c) Dyno-Mill KD 25						
ZrO2 beads 0.3–0.4 mm:						
1	102.5	35	85	13	90.6	95.7
2		120			77.7	83.7

values were very close to the r=0.997 reported above for the empirical Eq. 1.

We expressed degree of disintegration for each bead mill as power functions of operational parameters in a form:

$$\mathrm{Des}(\%) = a_0 Q^{n_1} \mathrm{BD}^{n_2} \mathrm{BF}^{n_3} \mathrm{PV}^{n_4} \mathrm{DW}^{n_5}, \text{etc} \quad (5)$$

where Q (kg h^{-1}) is feed rate; BD (mm) is beads diameter; BF (%) is beads filling of grinding chamber; PV (m s^{-1}) is peripheral velocity; DW (g l^{-1}) is algae dry weight; a_0, n_1–n_5 are empirical coefficients. The empirical correlations developed below can be used, e.g., for sensitivity analysis of the process parameters on the degree of algae cell disintegration in the bead mills investigated.

Influence of algae suspension feed rate, beads diameter, beads filling, peripheral velocity of discs mounted on stirring shaft, and algae dry weight on the degree of cells disintegration in the homogenizers Dyno-Mill KDL-Pilot A, KD 20 S, and KD 25 S, and reduction of bacteria count in the suspension of disintegrated algae cells was investigated (Table 3). For the homogenizer KDL-Pilot A, the data were correlated as:

$$\mathrm{Des}(\%) = a_0 Q^{n_1} \mathrm{BD}^{n_2} \mathrm{BF}^{n_3} \mathrm{PV}^{n_4} \mathrm{DW}^{n_5} \quad (6)$$

with estimated values of coefficients: a_0=1 748; n_1=−0.0356; n_2=0.326; n_3=0.0768; n_4=0.248; n_5=−0.763; correlation coefficient r=0.862.

For the homogenizer KD 20 S, the data (Table 3) were correlated as:

$$\mathrm{Des}(\%) = a_0 Q^{n_1} \mathrm{BD}^{n_2} \mathrm{BF}^{n_3} \quad (7)$$

where a_0=12.120; n_1=−0.224; n_2=0.0412; n_3=0.622; r=1.000.

For the homogenizer KD 25 S, the data (Table 3) were correlated as:

$$\mathrm{Des}(\%) = a_0 Q^{n_1} \quad (8)$$

with a_0=141.13; n_1=−0.125; r=1.000.

Homogenizer LabStar LS 1

Influence of the feed rate, beads diameter, frequency of stirring S (rpm), algae dry weight, number of passes N of algae suspension through the grinding chamber on the degree of disintegration was tested (Table 4). The data were correlated as:

$$\mathrm{Des}(\%) = a_0 Q^{n_1} \mathrm{BD}^{n_2} S^{n_4} \mathrm{DW}^{n_5} N^{n_6} \quad (9)$$

with a_0=63.42; n_1=−0.130; n_2=0.015; n_4=0.299; n_5=−0.413; n_6=0.138; r=0.926.

Homogenizer MS 18

Influence of the feed rate, beads diameter, beads filling, rotor peripheral velocity, algae dry weight, disintegrator gap size SG (mm), and number of passes N of algae suspension through the grinding chamber on the degree of disintegration was tested (Table 5). The data were correlated as:

$$\mathrm{Des}(\%) = a_0 Q^{n_1} \mathrm{BD}^{n_2} \mathrm{BF}^{n_3} \mathrm{PV}^{n_4} \mathrm{DW}^{n_5} \mathrm{SG}^{n_6} N^{n_7} \quad (10)$$

Table 4 Degree of cells disintegration and reduction of bacteria count in the homogenizer LabStar LS 1

Trial no	Algae dry weight (g L^{-1})	Feed rate (kg h^{-1})	Beads filling (% of chamber volume)	Stirring speed (rpm)	Degree of cells disintegration (%)	Reduction of bacteria count (%)
Glass beads 0.3–0.4 mm:						
1	69.4	5	85	3,000	95	98.6
2		10			93	96.9
3[a]		10			98–99	99.5
4[b]		10			98–99	99.2
Glass beads 0.5–0.7 mm:						
5	69.4	4.7	85	2,600	96	99
6		12		2,600	92	98.7
7		18		2,000	70	95.3
Glass beads 1.0–1.2 mm:						
8	69.4	5	85	2,800	93–94	96.6
9		9			91–92	92.6
Glass beads 0.5–0.7 mm:						
10	141.7	5	85	3,000	70	89
11		10			64	69.5
12[a]		10			71.5	91
13[b]		10			83	–

[a] Second pass of suspension
[b] Third pass of suspension

with a_0=1.641; n_1=−0.0729; n_2=0.191; n_3=0.386; n_4=0.261; n_5=0.891; n_6=−1.213; n_7=0.298; r=0.836.

Economics of a continuous disintegration process can be estimated, e.g., by specific energy consumption P/M, where P is energy consumption (kW) and M is mass flow (kg (dry weight) h^{-1}) of disrupted cells in the outlet of the suspension from disintegration chamber. The mass flow M was expressed as: $M=(Q/\rho_s)\,X\,(\mathrm{Des}\,(\%))/100$, where X is algae dry weight (g l^{-1}) in the feed and ρ_s (g l^{-1}) is suspension density (ρ_s=1,100 g l^{-1} was considered as an approximation). It is apparent from Table 6 that power consumption in the Dyno-Mill was little influenced by feed rate, but specific energy consumption (kWh per 1 kg (dry weight) of disrupted cells) decreased with increasing feed rate. On the other hand, energy consumption per 1 kg (dry weight) of disrupted cells increased with increasing degree of cells disintegration.

Experimental and estimated values of the degree of the cells disintegration for all homogenizers are plotted in Fig. 3.

Reduction of algae accompanying viable bacteria increased with increasing the degree of algae cells disintegration—Fig. 4.

Table 5 Degree of cells disintegration and reduction of bacteria count in the homogenizer MS 18

Trial no	Algae dry weight (g l^{-1})	Feed rate (kg h^{-1})	Beads filling (% of chamber volume)	Peripheral speed (m s^{-1})	Degree of cells disintegration (%)	Reduction of bacteria count (%)
Glass beads 0.5–0.75 mm; gap 6.5 mm						
1	110.6	18	75	13	83	99
2		18	75	15	96	99.8
3		18	80	15	90	87.1
4		26	80	15	87	–
ZrO2 beads 0.3 mm; gap 6.5 mm						
5	110.6	26	60	15	65	99
6		26	75	15	83	99.4
7		26	80	16	85	99.6
8		26	80	15	84	99.3
9		18	80	16	85	99.6
10		35	80	16	80	98.9
Glass beads 0.25–0.45 mm; gap 8.3 mm						
11	124.7	18	80	16	72.3	91.9
12		26		16	64.1	84.6
13		35		16	50.1	84.4
14[a]		35		16	83.9	96.4
15[a]		18		16	86.0	96.8
Glass beads 0.5–0.75 mm; gap 8.5 mm						
16	124.7	18	80	16	84.7	96.4
17		26		16	83.9	96.4
18		35		16	81.4	95.5
19[b]		35		16	90.1	97
20[b]		18		16	93.0	97.2
ZrO2 beads 0.3 mm; gap 8.5 mm						
21	124.7	18	80	16	69.8	91.3
22		26		16	66.9	86.6
23		35		16	45.5	72.3
24[c]		35		16	74.4	92.1
25[c]		18		16	75.6	100
Glass beads 0.5–0.75 mm; gap 8.5 mm						
26	87.7	18	80	16	42.9	70.4
Glass beads 0.5–0.75 mm; gap=6.5 mm						
27	87.7	18	80	16	76.7	100
28	125.1	18		16	89.4	100

[a] Second pass of suspension disintegrated before in trial numbers 11–13
[b] Second pass of suspension disintegrated before in trial numbers 16–18
[c] Second pass of suspension disintegrated before in trial numbers 21–23

Discussion

Batch disruption in bead mills can be described as a first-order process (Engler 1993). We verified this—Eq. 4 above—for *Chlorella* suspension re-circulated in the homogenizer Dyno-Mill ECM-Pilot. For continuous disruption of microbial cells, the first-order kinetic is maintained, but the residence time of the cells in the mill must be considered. The cells residence time T in a grinding chamber would be inversely proportional to the suspension feed rate Q: $T=k_2/Q$, where k_2 is an empirical coefficient. Considering Eq. 4 and taking $k_3=k_1k_2$, the influence of feed rate on the degree of disintegration can be expressed as:

$$\mathrm{Des}(\%) = 100[1 - \exp(-k_3/Q)] \quad (11)$$

From the data in Tables 3, 4, and 5, it was found for the individual homogenizers KDL-Pilot A, k_3=34.64; KD 20, k_3=71.71; KD 25, k_3=175.50; LabStar LS 1, k_3=18.94; MS 18, k_3=31.61; mean correlation coefficient between the all experimental and estimated Des (%) values, r=0.845. It is apparent that k_3 values widely differed for individual homogenizers. Thus, it may be expected that the influence of feed rate on disruption is more complex than a simple first-order relation (Řeháček and Schaefer 1977, Kula and Schütte 1983, 1987). The first-order rate constant depends on a number of parameters such as design and the speed of the agitator, bead loading, bead size, and cell concentration (Heim and Solecki 1999). To specify such dependence for breaking *Chlorella* cells, we computed k_3 from Eq. 11 for the known Q and Des (%) data given in Table 5 (the homogenizer MS 18). In this case, only the data for single pass of suspension through the bead mill were considered. Mean value± Std. dev. was: k_3=36.71±13.85, n=28. The great Std. dev. value indicates that k_3 may be indeed influenced by the above-mentioned parameters. We found the correlation: $k_3 = a_0 Q^{n_1}\mathrm{BD}^{n_2}\mathrm{BF}^{n_3}\mathrm{PV}^{n_4}\mathrm{DW}^{n_5}\mathrm{SG}^{n_6}$, with a_0=1.543; n_1=−0.113; n_2=0.159; n_3=0.463; n_4=0.185; n_5=0.891; n_6=−1.194; r=0.821. Values of the coefficients are close to these obtained for the MS 18 homogenizer in Eq. 10.

First-order disruption kinetics of baker's yeast in bead mills have been reported by many authors (Mogren et al. 1974, Chisti and Moo-young 1986) in homogenizers with predominant plug flow, whereas in homogenizers in which the rotor design permits significant backmixing the disruption deviated from the first-order behavior. The transport behavior of a homogenizer corresponds neither to plug-flow reactor nor to an ideal continuous flow-stirred tank reactor. The behavior of a given mill can be analyzed from tracer response curves.

The residence time distribution (RTD) of bead mills corresponds to a cascade of stirred tank reactors (Limon-Lason et al. 1979, Schütte and Kula 1990). The RTD is a function of feed rate—at high feed rates, backmixing is reduced compared to that at low feed rate and of stirrer geometry (Kula and Schütte 1987).

In our work, the degree of disintegration decreased with increasing feed rate (see Eqs. 6–10) where the coefficient n_1 ranged from −0.0356 to −0.224. Different values of n_1 may reflect different RTD of the cells in the bead mills investigated. The volume of grinding chamber determines the range of possible feed rates. The optimal value for a given microorganism depends on the agitator speed, bead loading, etc., and must be found experimentally. Generally, disruption of microbial cells decreases with increasing feed rate. Mogren et al. (1974) found for continuously working bead mill Dyno-Mill KD 5 (grinding chamber volume 5 l, electromotor 11 kW) that specific electrical energy requirement per kilogram of disintegrated dry substance (yeast cells) decreased (from 0.8 to 0.25 kWh kg^{-1}) with increasing feed rate (in the range 100–450 l h^{-1}) for yeast suspension 110 g (dry weight) l^{-1}. We found for the Dyno-Mill KD (Table 6 in this work) that at the feed rate of 120 kg h^{-1}, about 3 kWh per 1 kg (dry weight) of disrupted cells was needed. This is several times higher than it was reported by Mogren et al. (1974) for

Table 6 Mass flow M of disrupted algae cells from disintegration chamber, power consumption P and specific power consumption P/M in the homogenizers Dyno-Mill

Algae dry weight (g l^{-1})	Feed rate (kg h^{-1})	Beads filling (%)	Speed of agitator discs (m s^{-1})	Degree of cells disintegration (%)	M (kg (dry weight) $h^{-1)}$	P (kW)	P/M (kWh kg (dry weight)$^{-1}$)
a) Dyno-Mill KD 20 S							
ZrO2 beads 0.3–0.4 mm:							
124.2	35	85	13	82.8	3.27	27.4	8.37
	38	75		75.2	3.22	24.5	7.60
	120	75		58.1	7.87	24.5	3.11
ZrO2 beads 0.6–0.8 mm:							
124.2	35	85	13	85.2	3.36	25.1	7.51
b) Dyno-Mill KD 25 S							
102.5	35	85	13	90.6	2.95	29.6	10.03
	120			77.7	8.69	24.5	2.82

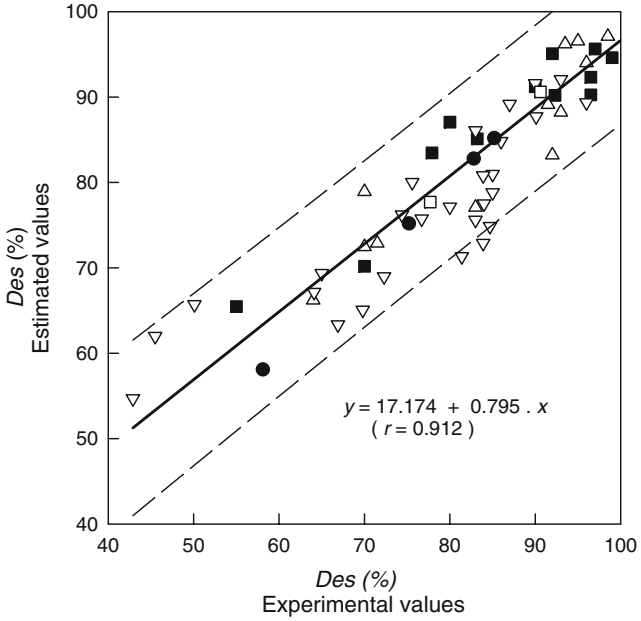

Fig. 3 Comparison of experimental and estimated (Eqs. 1, 6–10) values of the degree of cells disintegration *open circle* KDL-Pilot A; *filled circle* KD 20 s; *open square* KD 25 s; *filled square* ECM-Pilot; *open upright triangle* LabStar LS 1; *open downward triangle* MS 18 (*regression line* and *95% prediction intervals* are plotted)

yeasts. The difference may reflect the higher mechanical strength of the *Chlorella* cell wall. Schütte et al. (1986) recommend that for economic reasons the feed rate should be as high as possible, the power consumption being influenced only little. These findings are in accord with our results (Table 6).

Cell concentration had a strong influence on the degree of disintegrations: it decreased in the homogenizers KDL-Pilot A and LabStar LS 1 and it increased and in the homogenizer MS 18, with increasing cell concentration. This is in contrast with findings of Mogren et al. (1974) for yeast disintegration in a bead mill, who found that the cell concentration had only a small effect on disruption. The cell concentration in the feed should be as high as possible to minimize the power consumption per unit of disrupted cells mass (Mogren et al. 1974, Schütte and Kula 1990).

Disruption of microbial cells is performed with beads diameters ranging between 0.2 and 1.5 mm (Schütte and Kula 1990). Hard unleaded glass (density 2.5 g cm^{-3}) and zirconium oxide (density 5.4 g cm^{-3}) for cell breakage are generally used. Molina Grima et al. (2004) reported that degree of microorganism disintegration was increased by about 50% using zirconia–silica beads compared to glass beads, probably of their greater density. This finding was not supported in our work (see Table 5, trial numbers 11–13 vs. trial numbers 21–23). This may be explained by the fact that the high-density beads are advantageous for wet milling of high viscous suspensions, whereas in solutions of low viscosity, such as microbial suspensions, glass beads yield better results (Schütte and Kula 1990). The number of beads and the contacts between beads and the algae cells should increase and kinetic energy of the beads should decrease with decreasing beads diameter. However, there should be a lower limit to the beads diameter due to bead fluidization and sieve blinding problems encountered with very small beads (Chisti and Moo-young 1986). Optimal

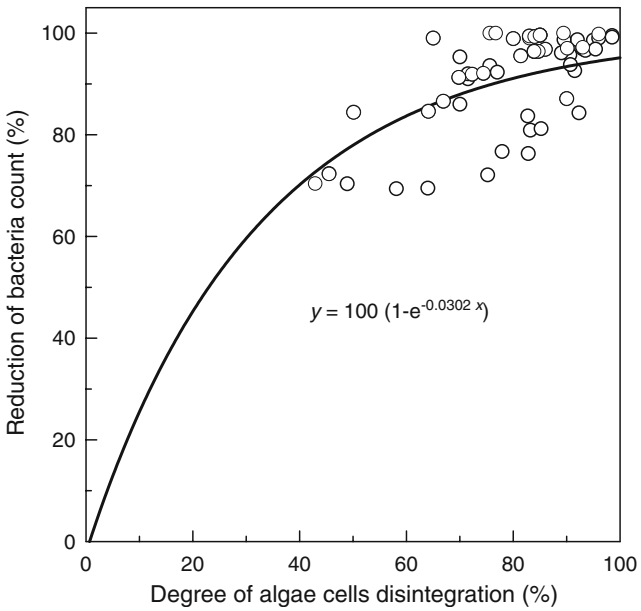

Fig. 4 Relationship between degree of algae cells disintegration and reduction of viable bacteria count

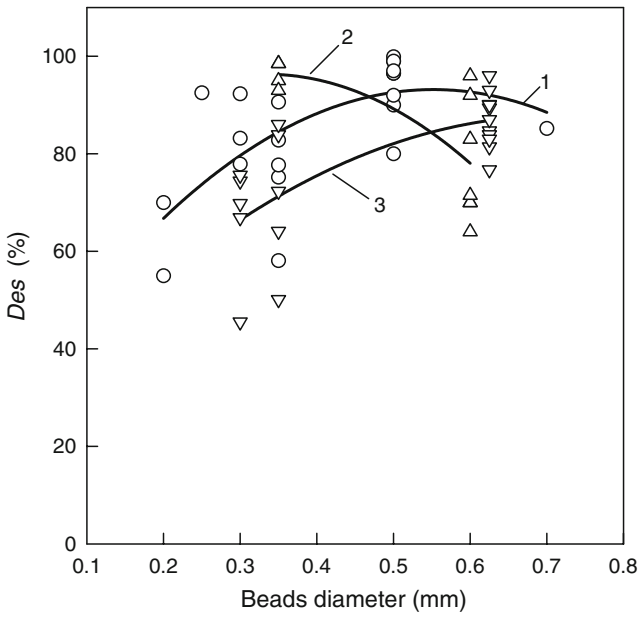

Fig. 5 Influence of beads diameter on the degree of cells disintegration 1 (*open circle*) KDL-Pilot A; KD 20 s; KD 25 s; 2 (*open upright triangle*) LabStar LS 1; 3 (*open downward triangle*) MS 18

bead size depends on agitator design and mill geometry and it may vary for different homogenizers. Our results (Fig. 5) indicate that optimum glass beads diameter for the homogenizers Dyno-Mill and LabStar LS 1 was 0.3–0.5 mm, for the MS 18 it was 0.5–0.7 mm. Hedenskog et al. (1969) found that a bead size of 0.35–0.50 mm gave the best disintegration value of the microalga *Scenedesmus obliquus* in a bead mill of the construction similar to the Dyno-Mill: at a flow rate 40 L h^{-1}, 90% of the cells were disrupted, compared to the beads of 0.5–0.7 mm diameter (80% of disrupted cells) and the beads of 1.1–1.2 mm (50% of disrupted cells).

It is apparent from Eqs. 6, 9, and 10 that the degree of cells disintegration increased with increasing stirring speed, the exponent n_4 varied only little (n_4=0.248–0.299) despite of different processing parameters and different homogenizers used. With increasing stirring speed the energy input will rise as well as the temperature and wear of the beads (Schütte and Kula 1986). The rate of disruption will increase with increasing stirring speed, but a simultaneous increase in dispersion in the grinding chamber may decrease this effect (Engler 1993). Less than 1% of the energy introduced by stirring can be used for the disruption of cells (Schütte and Kula 1990); the rest is converted to heat. Costs of cooling will decrease with increasing processing temperature. Not only a thermal denaturation of some heat sensitive algae cell components (vitamins, enzymes, etc.) may be a problem, but also denaturation caused by shear and other mechanical forces. In our tests, outlet temperature of algae suspension after passing through the bead mills grinding chamber did not surpass 35°C, which can be considered optimal for preserving valuable compounds of the cells.

In our work, degree of disintegration increased with increased beads filling volume of disintegration chamber, the highest value of beads filling investigated was 85%. If the bead filling is too low, shear forces and frequency of collisions will not be sufficient to provide good disintegration. If the bead filling is too great, interference among the grinding elements prevents the establishment of effective velocity profiles. Likewise, temperature and power consumption increase markedly with increased beads filling.

Algae cultures grown in outdoor bioreactors contain accompanying bacterial microflora. Number of viable bacteria in the product which is disintegrated and spray dried algae biomass used mostly as an additive in human diet, should not surpass allowable limits. Decrease of the bacteria count in the product can be achieved by: (1) repeated washing of thickened algae suspension using continuous separators; (2) passing of thickened algae suspension through mechanical homogenizers, as investigated in this work. Bacteria have the size about 0.5–1 μm cell diameter, which is approximately 1/10–1/5 of the average *Chlorella* cell size. A two-order decrease of bacteria count by their passing through the homogenizers was found in our experiments.

Acknowledgements We thank the institutions in which the experiments were performed: Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland; Netzsch Feinmahltechnik, Selb, Germany; Pro-Tech laboratory, Neuenburg, of the FrymaKoruma, Rheinfelden, Germany.

We also thank the Czech representatives of these institutions for valuable cooperation.

References

Becker EW (1994) Microalgae—Biotechnology and microbiology. Cambridge University Press, 293 p

Doucha J, Lívanský K (1995) Novel outdoor thin-layer high density microalgal culture system: Productivity and operational parameters. Arch Hydrobiol 106/Algolog Stud 76:129–147

Doucha J, Lívanský K (1998, 1999): Process of outdoor thin-layer cultivation of microalgae and blue-green algae and bioreactor for performing the process. Greek Patent 1002924, 1998; US Patent 5981271 A, 1999

Doucha J (1998) The *Chlorella* program in the Czech Republic. Inst. Microbiol., Czech Acad. Sci., 16 p

Doucha J, Lívanský K (2006) Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in Middle and Southern European climate. J Appl Phycol 18:811–826

Engler CR (1993) Cell breakage. In: Harrison RG (ed) Protein Purification Process Engineering. CRC Press, pp 37–55

Chisti Y, Moo-young M (1986) Disruption of microbial cells for intracellular products. Enzyme Microb Technol 8:194–204

Hatti-Kaul R, Mattiasson B (2003) Release of protein from biological host. In: Hatti-Kaul R, Mattiasson B (eds) Isolation and Puirification of Proteins. CRC, pp 1–28

Hedenskog G, Enebo L, Vendlová J, Prokeš B (1969) Investigation of some methods for increasing the digestibility in vitro of microalgae. Biotechnol Bioeng 11:37–51

Heim A, Solecki M (1999) Disintegration of microorganisms in a bead mill with a multi-disc impeller. Powder Technol 105:389–395

Kula MR, Schütte H (1983) Experiences with a 20 litre industrial bead mill for the disruption of microorganisms. Enzyme Microb Technol 5:143–148

Kula MR, Schütte H (1987) Purification of proteins and the disruption of microbial cells. Biotechnol Progr 3:31–42

Limon-Lason J, Hoare M, Orsborn CB, Doyle DJ, Dunnill P (1979) Reactor properties of a high-speed bead mill for microbial cell rupture. Biotechnol Bioeng 21:745–774

Mendes-Pinto MM, Rapovo MFJ, Bowen J, Zouny AJ, Morais R (2001) Evaluation of different cell disruption processes on encysted cells of *Haematococcus pluvialis*: effects on astaxanthin recovery and implications for bio-availability. J Appl Phycol 13:19–24

Middelberg APJ (1995) Process-Scale Disruption of Microorganisms. Biotechnol Adv 13:491–555

Mogren H, Lindblom M, Hedenskog G (1974) Mechanical disintegration of microorganisms in an industrial homogenizer. Biotechnol Bioeng 16:261–274

Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Vhosti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Advances 20:491–515

Molina Grima E, Acién Fernández FG, Robles Medina A (2004) Downstream Processing of Cell-mass and Products. In: Richmond A (ed) Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell, pp. 215–252

Mölls HH, Hörnle R (1972) Wirkungsmechanismus der Nasszerkleinerung in der Rührswerkkugelmühle. In: Rumpf H, Schönert K (eds) Dechema-Monographien No 1292–1362 (69), Teil 2. Verlag Chemie, Weinheim, p. 631–661

Novotný P (1964) A simple rotary disintegrator for micro-organisms and animal tissues. Nature. Apr 25; 202:364–366

Řeháček J, Beran K, Bičík V (1969) Disintegration of microorganisms and preparation of yeast cell walls in a new type of disintegrator. Appl Microbiol 17:462–466

Řeháček J (1971) Continuous disintegration of microorganisms in a new laboratory apparatus. Experientia 27:1103

Řeháček J, Schaefer J (1977) Disintegration of microorganisms in an industrial horizontal mill of novel design. Biotechnol Bioeng 19:1523–1534

Schütte H, Kula MR (1986) Einsatz von Rührwerkskugelmühlen und Hochdruckhomogenisatoren für den technischen Aufschluss von Mikroorganismen. Biotech-Forum 3:68–80

Schütte H, Kraume-Flügel R, Kula MR (1986) Scale-up of mechanical cell disintegration: Influence of the stirrer geometry on the residence time distribution in a 20 L high-speed bead mill. Ger Chem Eng 9:149–156

Schütte H, Kula MR (1990) Bead mill disruption. In: Asenjo JA (ed) Separation Processes in Biotechnology. Marcell Decker, pp. 107–141

Wimpenny JWT (1967) Breakage of microorganisms. Proc Biochem 2:41–44