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Measurement of bubble and pellet size distributions: past and current image analysis technology

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Abstract Measurements of bubble and pellet size distributions are useful for biochemical process optimizations. The accuracy, representation, and simplicity of these measurements improve when the measurement is performed on-line and in situ rather than off-line using a sample. Historical and currently available measurement systems for photographic methods are summarized for bubble and pellet (morphology) measurement applications. Applications to cells, mycelia, and pellets measurements have driven key technological developments that have been applied for bubble measurements. Measurement trade-offs exist to maximize accuracy, extend range, and attain reasonable cycle times. Mathematical characterization of distributions using standard statistical techniques is straightforward, facilitating data presentation and analysis. For the specific application of bubble size distributions, selected bioreactor operating parameters and physicochemical conditions alter distributions. Empirical relationships have been established in some cases where sufficient data have been collected. In addition, parameters and conditions with substantial effects on bubble size distributions were identified and their relative effects quantified. This information was used to guide required accuracy and precision targets for bubble size distribution measurements from newly developed novel on-line and in situ bubble measurement devices.

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List of symbols

a_{32}	interfacial area, surface area per unit volume, $1/\mu$
A	area of object
A_c	experimentally determined constant in Calderbank equation (Eq. 20)
A_3	skewness of distribution
A_4	kurtosis of distribution
AR	aspect ratio, longest to shortest diameter
C	circularity, 1/SF (Eq. 5)
C_v	coefficient of variation
d	equivalent spherical bubble diameter
d_a	sample mean bubble diameter, arithmetic mean (Eqs. 9, 11–13)
d_F	Feret diameter (Eq. 6), diameter of equivalent circular object with same area as irregularly shaped object
d_g	log-geometric mean diameter (Eq. 3)
d_i	diameter of bubble i
d_{long}	longest diameter of a single circular object
d_{max}	maximum stable bubble size; maximum bubble diameter
d_{min}	minimum bubble diameter
d_{short}	shortest diameter of a single circular object
d_{30}	volumetric mean diameter (Eq. 4)
d_{32}	Sauter mean diameter (Eq. 2)
d_{50}	median value of diameter; diameter for which normalized cumulative volume curve is 0.5

d_{99}	diameter that is larger than 99% of all diameters in the cumulative number distribution of bubbles
K_La	volumetric gas–liquid mass transfer coefficient
N	impeller speed
n	total number of bubbles, sample size
n_i	number of bubbles of diameter d_i
P	perimeter of object
P/V_L	power input to dispersion per unit liquid volume (gassed power)
Q	volumetric gas flow rate
R	roundness
SF	shape factor (Eq. 5)
V_b	total volume of bubbles
β	experimentally determined constant in Calderbank equation (Eq. 20)
γ	experimentally determined constant (Eq. 21)
δ	experimentally determined constant (Eq. 23)
ρ_c	liquid (continuous phase) density
ρ_g	gas (dispersed phase) density
ρ_p	pellet density
Φ	void fraction of dispersed phase (hold up) (Eqs. 16 and 20)
ε_T	gassed power input per unit mass (Eq. 17)
μ_G	gas viscosity
μ_L	liquid viscosity
σ_a	standard deviation from arithmetic mean
σ_g	log-geometric mean standard deviation
σ_T	surface tension

Abbreviations

BSA	Bovine serum albumin
CCD	Solid state charge-coupled device cameras, two-dimensional, self-scanning, electronic analog imaging device
CC-TV	Closed circuit television, standard camera equipment
Chalnicon	Sensor tube that has cadmium selenide-based target layer for face plate material
DAT	Data acquisition time
EC	Electronic commerce
fps	Frames per second
IPS	In-plane-switching, technology to produce high-quality LCDs
LED	Light emitting diode
MAT	Measurement acquisition time
NTSC	National Television System Committee, 525 lines, 30 Hz (Americas and Far East)
PAT	Process analytical technology
PC	Personal computer
RW	Read/write
SVHS	Super VHS (vertical helical scan), enhanced quality and higher horizontal resolution

Introduction

Uses of measurements for bubbles and pellets

Accurate and representative bubble and pellet size distributions have been used to characterize biochemical processes. Fermentation of industrially important fermentation products involves multiphase dispersion [110], focusing primarily on gas in liquid systems. Gas bubble size depends on (1) the type of sparger configuration (i.e., point, ring, or frit) and its position relative to the impeller, (2) bioreactor operating conditions such as shear (affected by agitator speed/velocity and type), volumetric air flow rate, and temperature, and (3) gas/liquid properties such as the type of media (affected by density and viscosity) and the presence of surface-active components (i.e., surface tension) [44, 67, 92]. Bubble sizes range considerably with some reported to be up to around 5–10 mm in bubble columns containing viscous solutions [59]. Bubble size distributions vary widely within a stirred tank based on the distance from the impeller [6]. In addition, bubbles change in size over the course of the fermentation. Quantification of bubble sizes is important to establish mass transfer characteristics (based on gas–liquid interfacial area) when oxygen transport to cells across gas–liquid interfaces becomes a limiting factor. In these situations, there is a direct influence of bioreactor parameters on culture yields [110], and thus it is useful to reliably quantify bubble size.

Several industrially important cultures grow as multi-cell pellets (vs. filamentous mycelia) for maximum productivity and lower viscosity. Pellet sizes range from 40 to 1,000 μm for *Penicillium chrysogenum* [34, 35, 58] and 400 to 2,500 μm for *Streptomyces tendae* [109]. Pellet sizes depend not only on the agitator shear, but also on several other factors such as culture and media [40, 73]. Optimal pellet size avoids nutrient limitations associated with larger pellets which cause cell death and reduced productivity [82]. Thus, quantification of pellet sizes is important to identify transport restrictions (based on pellet diameter and density) when diffusion in the presence of nutrient uptake limits radial penetration of nutrients including oxygen [20]. Interestingly, the surrounding turbulence improves nutrient transport in pellets [113].

In situ versus sampling; on-line versus off-line

On-line measurements are continuous in nature and can be based either on continuous sampling from the bioreactor system or direct sensing. The sample size for on-line analysis is larger; thus more representative data are generated in more timely intervals and greater quantities of data accumulate faster with little manual intervention [19]. On-line analysis should reduce and not increase the laboratory workload [19]. There has been a perceivable trend towards computer-controlled chemical operating plants [47], and most recently process analytical technology (PAT), in part based on on-line sensors, is being applied to many processes [93].

In situ sensing avoids the instrument being limited by removal of a sample as it can be difficult to obtain representative samples due to operator variability and sampling inconsistencies [19]. In situ analysis also avoids sample alteration caused by removal from the processing environment [19]. It omits error caused by sample preparation. Specifically for mycelia undergoing image analysis, sample preparation errors can be ± 8% [71], and dilution of particles inappropriately can alter particle shape [19]. Furthermore, biomass dilution factors must be optimized; samples of mycelia typically are diluted 100-fold prior to image analysis [57]. Over-dilution increases the number of objects detected, while under-dilution causes overlaid objects; optimal dilution is the lowest dilution that enabled the maximum percentage of recognition [5]. As the overall variance of analysis is composed of the sum of individual variances of each step, a 10% sampling error is large compared with a 1% standard error in size analysis [47]. Errors also exist when the sensor is placed next to the glass window of a tank (applicable primarily to small-scale laboratory apparatus) which can affect measurements due to its curvature, although this error is smaller than the sampling error. In situ methods in which the probe is inserted into the process avoid this problem, but an inserted probe can potentially obstruct bubble position or modify bubble shape [91].

Background

Historical and currently available measurement methods

General

The present state of the art in particle size analysis is characterized by the use of optical methods [47]. Particle size has been measured using several methods such as low-angle laser light scattering, ultrasound, optical image analysis, and direct mechanical measurement; each method results in accurate particle size data within its intended set of parameters [19]. There are four common methods for bubble analysis: photographic, electrical conductivity, electro-optical, and light scattering [10]. The focus of this review is on-line photographic methods using optical image analysis for bubbles size analysis. However, there are far more published studies using image analysis for pellet and morphology than bubbles and applying image analysis to off-line rather than on-line samples. In fact, image analysis is well established for quantifying and characterizing mycelia from off-line samples of fermentation systems [77]. Thus, surveys of past and present image analysis techniques for both bubbles and pellets (morphology) were conducted and evaluated to assess issues and trends relevant to the development of new instrumentation devices.

Early methods of pellet size analysis utilized manual sieves with various mesh sizes [34, 35, 109]. Morphological characterization of filamentous organisms (free cells, mycelia, and pellets) in submerged culture has been significantly enhanced by image analysis technology developments [24]; some of these developments have also been applied to bubble size quantification. Consequently, bubble quantification also has progressed significantly from initial methods that used simple manual measurements of photographs [88]. Many cell imaging systems are stagnant and most bubble systems are dynamic, however. All systems need the ability to focus automatically or manually to obtain a clear image [70]. Costs for fully automated image analysis can be too high for certain applications, however, and additional manual steps can be required that are time-consuming [36].

Early photographic methods

Compared with conductivity, light scattering, and electro-optical methods, the photographic method is the most tedious, but it handles the broadest bubble size distribution and is the most reliable for viscous media [76]. It is one of the best methods for obtaining gas bubble surface area despite the tedious effort required [101]. Overall, photography is more sensitive to smaller bubbles than larger ones, requires relatively clear media, and possesses issues with occlusion and depth of field [76]. Cameras have been attached to specially adapted microscopes located externally to the process apparatus or placed directly adjacent to the apparatus wall with resulting images printed and analyzed after viewing through a microscope [13].

Manual counting and analysis techniques for photographs or videos are laborious, tedious, imprecise, and time-consuming which make them hard to be reliably quantitative [5, 110]. Early photographic methods were manual in nature, performing measurements directly from the microscope stage with the use of a micrometer or indirectly using photographs [77]. Initial investigations of mycelial morphology relied upon these inaccurate and time-consuming manual measurements from photographs [105]. An electronic digitizer increases speed [77], but digitizing tablets are operator-dependent and slow [105]. Digitizing methods also have the disadvantage of being labor-intensive and time-consuming [1] and are not particularly precise or accurate [70].

Image acquisition, processing, and analysis methodology

An on-line, automated system should produce data within a process-relevant timeframe. This time is evaluated through quantification of the (1) data acquisition time (DAT) or time to acquire a single piece of data and (2) measurement acquisition time (MAT) or time to acquire sufficient data to produce an accurate result and reset the system for the next measurement. Ideally the MAT is equal to the DAT [19]. The absolute value of the MAT is most relevant. If the MAT is greater than or equal to the process time constant, the instrument is unable to resolve process temporal changes. If the MAT is less than the process time constant, then only non-quantitative process trending can occur. If the MAT is much less than the process time constant, the instrument can observe process behavior and upsets [19] and thus be useful for real-time monitoring and control applications. Specifically for particle size counters, the MAT is much greater than the DAT [19]. For microbial secondary metabolite or animal cell processes, MATs of no faster than 1–2 per hour are likely to be sufficient; for faster metabolizing *Escherichia coli* or yeast fermentations, higher MATs of up to 4–6 per hour might be required.

Measurement systems utilize a television camera mounted on a microscope with a video signal of the field of view sent to a computer capable of image processing and analysis. Tube cameras offer high sensitivity especially in low light levels [77]. The image is digitized in both space and tone to produce pixels (picture elements), each of which is assigned a grayness level. Further image processing is done to improve quality, and then images are analyzed to obtain measurements [70]. Conventional video cameras can be synchronized with the flashing of a strobe light to speed up acquisition to avoid blurred images [99], and this technique avoids use of an expensive fast acquisition digital or analog video camera [110]. Sharp images are obtained using either stroboscopic imaging or shutter speeds less than 0.001 s, but these fast shutter speeds require a lot of light [89]. Alternatively, high-speed video cameras may be utilized [99].

Typical hardware consists of high-quality cameras (cine-photographic equipment). The quality of most cameras has a specification of a 49 dB signal-to-noise ratio which translates into 7 bits of real information and 1 bit of noise [89]. Owing to noise and degradation with older cameras, previously there were only about 64 gray levels actually distinguishable in data, compared to about 30 levels in the human eye [89]. Images can be divided into pixels (e.g., 640 $\times$ 480 pixels) with each pixel having 256 brightness levels [89], and current cameras have 256 gray levels as well when used in the 8 bit mode. A calibration factor is required depending on the magnification to convert the inter-pixel distance to microns [70]. There is a ''real-time'' DAT, for example 30 frames/s (fps) [89], which can be increased if the number of pixels per frame (i.e., resolution) decreases. For high-resolution imaging, an analog signal path for data is avoided; a digital signal path reduces degradation and improves resolution [89].

Basic flow charts are similar for various image analysis systems. They consist of the following common stages: initialization/set up/autofocus, image capture/detection, image optimization/enhancement, segmentation, image processing (sometimes with manual editing), measurement/calculation, archive/file storage, and evaluation/analysis [1, 23, 77]. There is a need to set up (1) the hardware's focus, brightness, and calibration parameters and (2) the software's image processing parameters [77]. Segmentation, separation of the image into objects of interest to be measured and background, is an important step between image processing and image analysis; it distinguishes using relative brightness [23, 89]. The threshold value delineates the objects from the background [89], with all pixels brighter (i.e., grayer) than a preset value of interest [16, 70]. Real-time gray level differences between two successive frames are employed to detect moving objects and subtract out stationary objects; the length of the delay between two frames is chosen to avoid image overlapping [33]. Erosion removes pixels from an image that should not be there, and dilation adds pixels to an image [89], respectively, decreasing and increasing an object's size along its boundaries. A masking binary (i.e., black and white) image is defined which shows which objects have been selected for further processing, and subsequent processing is based on this binary

image [1, 70]. Sometimes the criterion that there are no holes in circular objects (i.e., regions of background) is applied when selecting objects of interest [70]. Several other types of image processing filters can also be applied to raw images prior to analysis.

Automation of image analysis makes it independent of the operator and faster [71]; automated quantification also avoids bias by the observer [77]. Image analysis is more precise than the digitizing tablet method [1] and has replaced digitizing tablets in many applications. Digital image processing has greater speed and better size resolution and avoids manual steps, so throughput is improved [82]. Input devices (such as a microscope or macroviewer with video camera, video recorder, and scanner) are interfaced with a high-performance PC with image processing microprocessors designed for speed [77]. The PC can store raw images for later recall, but storage requirements need to be reasonable [36]. Storage limitations have been partially overcome by today's more advanced, high-capacity hard disks. Technological advances and decreased computational costs have permitted quantitative image analysis to be used for process monitoring [5]. The use of manual editors in automated image analysis systems slows down processing, however [70].

Literature examples of bubbles size distribution methodologies

Table 1 shows characteristics of selected published photographic techniques used for obtaining bubble measurements and of selected non-photographic techniques which were included for comparison purposes. A review of these techniques reveals trends and progressions which when summarized clearly indicate key preferences in technology. All dispersions are gas (discontinuous phase)/liquid (continuous phase) unless otherwise stated. Bubble measurements began as fully manual in the mid-1950s and progressed through various levels of automation, frequently incorporating substantial manual steps to assure objects were selected appropriately. These measurement systems were located externally to the process. Most of them involved measuring only bubbles visible at the outside wall of a transparent vessel. Illumination often was by external flash using shutter speeds ranging from 0.0005 to 0.002 s or an internal strobe light synchronized to 50–100 fps. In a few cases, in situ microscopy was used. Magnification ranged from 3 to over 100-fold, with higher values used to discern liquid drops and solid particles. Curvature effects were quantified using internal standards, and in some cases they were minimized by placing cylindrical vessels and pipes into rectangular boxes filled with the dispersion's continuous phase. Some authors have identified measurement limitations at higher gas hold-ups and interferences due to additional light absorbing/scattering components of the dispersion. No one camera or image analysis software system has emerged as the standard; rather a wide variety of commercial hardware and somewhat customized software has been utilized.

Literature examples of cell, pellet, and morphological distribution methodologies

Table 2 shows characteristics of selected published photographic techniques used for obtaining pellet size and mycelial morphology measurements. A review also reveals trends and progressions, as well as similarities and differences to the task of bubble measurement. Techniques range from manual to digitized to semi-automated and then fully automated analysis. In some cases, manual interaction was initially used to classify images. Dilution from 2 to 800-fold was necessary to obtain distinct objects without overlap. Illumination and shutter speed generally were not reported as broth samples primarily were fixed and still shots from a microscope stage were most common. Magnification ranged from 2 to 200-fold for pellets which was about the same as for bubbles and up to 2,000-fold for mycelial hyphae measurements. No single type of image analysis software was used, but commercially available software often was highly customized.

Measurement assumptions and trade-offs

Key parameters of past and current photographic measurements systems are summarized in Tables 3 and 4 for bubbles and pellets, respectively, Ranges of these parameters bracket expected minimum requirements for future novel measurement devices. The size and number of objects used for each measurement vary considerably depending on the research application. For bubble measurements (Table 3), the size of the objects ranged from 40 μm to 20 mm, with most studies covering the range from 40 μm to 2 mm. The number of objects measured ranged from 50 to 4,000, with over half of the studies using between 480 and 1,000 objects. Previously, between 300 and 1,000 objects have been found to result in a stable bubble distribution in bubble column measurements [18]. There was a large variation (2–400) in the number of frames required for a single measurement. Measurement errors ranged from < 2

Table 1 Selected photographic and non-photographic bubble measurement systems from the literature

Photographic bubble measurement systems

Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References
Agitated glass vessel	Not given	Not given	0.0002 s repeating flash/not given	Manual measurement of photographs	[88]
Transparent bubble column	Reflex camera with single lens	3–5 × /scale	Flash/not given	Manual measurement of photographs; square column to avoid optical distortion	[2]
Bubble column	Camera with telephoto lens (Nikon)	Not given	Flash/0.0045–0.005 s	Semi-automatic particle analyzer (Zeiss); parallel optical windows mounted on column to avoid optical distortion	[10]
Bubble column	Not given	Not given	Flash/0.001	Semi-automatic particle analyzer (Zeiss)	[26]
Agitated transparent vessel	Not given	3–10 × via negative enlargement/not given	Flash/not given	Measurements from projected negatives	[100, 101]
Cylindrical plexiglass airlift column	SLR camera (Vivitar)	Not given/grid marks	Not given/ASA 1600	Four optically flat glass panels situated along column; Omicron Image Analysis system (Bausch and Lomb)	[29, 76]
Glass bubble column	Not given	Not given/graduated scale	Not given	Well-focalized bubbles in the center of column, direct measurements from photographs, diameter of sphere of equivalent volume to assumed ellipsoid shape	[46]
Transparent airlift bioreactor	Camera with close up lens (Nikon)	Not given	Not given	Image analyzer (Optomax)	[22]
Plexiglass bubble column	Video camera	Not given	Backlit/not given	Analyzed data on video monitor; major/minor axes measured; ellipsoid shape assumed; circulating external jacket to eliminate optical distortion	[59]
	Manual camera	Not given	Backlit/not given	Photographic negatives enlarged	[60]
Transparent bubble column	High-scan video camera (Proxitronics)	Not given	Fiber optic probe/not given	Kappa video scale on TV monitor with VHS recorder	[91]
Flow through a pipe (air in oil)	Camera plus mirror (instead of two cameras) (Nikon)	Not given	Not given/0.0005 s	Matching of images from side and front camera views using geometrical relationships; pipe enclosed in rectangular box filled with same fluid to avoid curvature effects	[53, 94]

Table 1 continued

Photographic bubble measurement systems					
Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References
Flotation cell	CCD camera; high-speed video recorder (Sony)	Not given/wire of known size	2 × 500 W lights/0.002 and 0.0017 s	Images printed and exposed to stereomicroscope camera (Olympus); image analysis system (Leco)	[13]
Transparent bubble column	CCD camera	Not given	Diffuse back light (halogen head lamp)/ not given	Image processing PC	[92]
Agitated glass vessel	SVHS video camera; stereomicroscope	Not given	Fiber optic strobe/ not given	Digitized and processed by computer; 3 points on bubble perimeter measured; shutter frequency synchronized for sharpness	[55]
Agitated glass vessel	Video camera; stereo-microscope	Not given	High-energy strobe inside vessel/not given	Strobe light intensity/frequency adjusted for sharpness and some indication of mycelia; 3 points on bubble perimeter measured	[27]
Agitated glass vessel with gas/oil/water dispersions	Manual 35 mm camera w/auto-macro lens (Olympus)	Not given/internal probe of known diameter	External light/0.001 s	Semi-automatic optical image analyzer (Optomax), manually indicated diameters; vessel curvature < 4%	[38, 39]
Agitated vessel with liquid/liquid dispersions	Video camera (Panasonic); zoom microscope (Olympus)	20–110 ×/not given	0.0005–0.0083 s strobe/50 fps	High-resolution SVHS video recorder with digitizing facilities; 3 points on drop perimeter identified by software (Panasonic)	[68, 69]
Plexiglass bubble column with gas, viscous liquid, and solid dispersion	Not given	35 × via enlarged photographs/internal reference mark	Not given	Not given; solid concentrations < 0.08 to avoid covering too large a bubble fraction	[42]
Agitated stirred tank	Dual CCD cameras	Not given/bubble gel	Laser perpendicular to camera/not given	Particle image velocimetry; vessel enclosed in rectangular container filled with water	[45]
Sparged downflowing liquid in pipe	Digital camera (Olympus)	Not given	Halogen/1,000–3,000 s^{-1}	Manual bubble tracing after using software (Image Pro Plus, Media Cybernetics) to process and enhance photographs; pipe enclosed in rectangular box	[95]

Table 1 continued

Photographic bubble measurement systems

Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References
Oil/water/gas dispersion in fungal broth in transparent vessel	Revolving nose piece of stereomicroscope (Olympus); color NTSC digital video camera (Hitachi)	1.8–11 × /not given	Submerged stroboscope probe (Optoelectronics)/60 Hz vertical scanning frequency	Commercial software (Image Pro Plus, Media Cybernetics) used to digitize images, basis for developed system; square external tank filled with water to avoid curvature effects	[52, 83, 99]
				Preprocessed and reduced to arc segments one pixel wide; Hough transform (method for linking points into defined shapes) used to detect objects	[110]

Non-photographic bubble measurement systems

Application	Sampling	Measurement and analysis method	References
Bubble column	Automatic syringe pump generated single air bubble in collector	Diameter calculated by manually counting total number of bubbles generated and total gas volume sparged; spherical bubble shape assumed	[54]
Floatation cell	Bubbles drawn up capillary under vacuum	Bubble length and velocity monitored by light intensity changes measured by 2 pairs of photo transistor-LED detectors; data capture system with processor and programmable timer	[67, 84]
Airlift bioreactors	Dispersion sample stream sucked into transparent capillary; bubbles transformed to slugs with length proportional to volume	Photoelectric probe; recorded by 2 light barriers using multi-channel analyzer; based on optical sensing (refraction/reflexion and absorption)	[9]
Agitated stirred tank	Bubbles drawn up capillary under vacuum	Pair of LED phototransistors; phototransistor switching time of a few hundred microseconds	[6, 7, 30, 31]
Bubble column/airlift bioreactors	In situ	Ultrasound reflection; single probe sends short pulse, then detects reflected signals from bubbles, mean value of reflected ultrasound power proportional to bubble surface size and number density and thus related to specific interfacial area	[11, 51, 98]

up to 10–20%, and they were not quantified for half of the studies. The best resolution reported was ± 0.05 mm [92]. MATs for automated acquisition and analysis were also not quantified often, but available reports ranged from 5 to 60 min.

For pellet and mycelial morphology measurements (Table 4), the size of the pellets ranged from 140 μm to 6.7 mm, a range similar to bubble measurements. The size of the hyphae typically measured ranged up to 1,800 μm, also similar to the bubble size measurement range. The number of objects per measurement ranged from 10 to 24,000 with over half of the studies using between 100 and 500 objects. The number of frames measured ranged from 5 to 236. Measurement errors varied widely, from 2 to < 27%. Resolution ranged from 1–2 μm/pixel [105] when used for hyphae length to 10–40 μm/pixel [24, 25]. MATs ranged from 10 to 170 min with shorter times of 3.6–10 s for measurements of particle flow velocity.

For small particle size measurements, the typical image analyzer acquired 250,000–500,000 images for one particle size distribution which required up to 10–20 min on some systems [19]. These values were much higher when compared to bubble size or pellet distribution instrumentation for which only hundreds of objects were measured to obtain statistically valid results [77]. Few researchers provide data in which the measurement sample size has been extended until differences in the size distribution parameters diminished into the noise/error range. The required number of objects per measurement becomes important if all images need to be reviewed/saved to evaluate accuracy of data or if it extends the MAT.

In obtaining a satisfactory number of bubbles in a single frame, a compromise existed as too many bubbles caused overlap and too few bubbles required counting many frames [13]. The number of frames that required counting for accurate data analysis decreased with lower mean bubble size [55]. Some measurements have been limited to void fractions < 10% to avoid large amounts of overlapping bubbles [53] or solid volume fractions < 0.08 to avoid particles covering too much of the bubble area at high solids loadings [42]. Similarly pellets in broth have been diluted so that the number of pellets in the area of analysis filled only 10–15% of the total image area [85, 86]. In addition, the area of picture with the lowest interference has been selected manually and clumping pellets (*P. chrysogenum*) were divided manually prior to further processing [82].

There was a trade-off between the elapsed time required for the system to reach steady state after operating conditions were changed and the number of different operating conditions that may be analyzed in a given time period. This time to reach steady state has ranged from 2–5 min in agitated gas–liquid systems [42, 55] to 30 min for oil–water–gas systems [83]. Another trade-off was the size range selected for bubble measurement since bubbles smaller than 1,000 μm generally were spherical and bubbles above 2,000 μm began to become non-spherical [55]; even larger diameter bubbles were highly irregular in shape. Larger bubbles required a greater number of frames to attain the statistically desirable number of items to be measured.

Measurement interferences

Common measurement interferences have been identified by prior researchers. One of the main problems monitoring multiphases in a bioreactor is acquiring clear images in motion [99]. The ability to distinguish bubbles from the background, analyze contiguous bubbles (bubbles touching, in front of, or overlapping other bubbles) either by exclusion or deconvolution, and omit large, irregularly shaped bubbles are also key factors. Size analysis under high gas hold-up conditions was complicated by bubble overlap and inability to clearly distinguish individual bubbles [45]. In a few specific instances, bubbles positioned near a huge bubble swarm were incorrectly included within the swarm by the imaging software. An estimate of gas hold-up is obtainable by quantifying the clear areas of an image comprised of bubbles and bubble swarms.

The effect of broth turbidity on the depth of field and interference by cell solids has been another limitation of optically based methods. The presence of protein decreases image contrast [83] by blurring object edges. In addition, particles do not transmit light as bubbles do. In one application, acceptable bubble images (i.e., objects with dark edges and a shiny middle) and an indication of dispersed biomass between bubbles were obtained only up to 5 g/L biomass dry cell weight [27]. Reflection from stainless steel tank internals (i.e., impeller, agitator shaft, sparger) can also interfere, resulting in bright blotches which are reduced in the presence of medium and cells.

Distribution calculations from data

Specific mathematical equations used for bubbles sizes

Size measurements are obtainable based on direct measurements of diameter, area, or volume, or using back-calculations to obtain an equivalent diameter assuming a spherical shape. There are several

Table 2 Selected photographic cell, pellet, and morphological measurement systems from the literature

Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References (culture genus)
Morphology in broth samples	CCD camera; Optiphot Microscope (Nikon)	2000 × /not given	Not given	Manual image analysis system; PC with frame grabber; image analysis software (Image House)	[12, 65, 66] (*Penicillium*)
Morphology in broth samples diluted 100 ×	Photographs via microscope	Not given	Not given	Projected photographs using adapted microfilm reader onto an electronic digitizer; custom PC analyzed data	[57] (*Penicillium*)
Morphology in broth samples diluted 20 ×	Camera (Olympus); Optiphot microscope (Nikon)	100 × /graticule slide	Not given	Digitizer (Hewlett Packard); overlapping microorganisms rejected	[1] (*Streptomyces*)
Broth samples	Video camera images traced onto acetate sheets	Not given/stage micrometer	Not given	Pellet outlines digitized using digitizing tablet (Hewlett Packard); radius back-calculated assuming spherical pellets	[103] (*Streptomyces*)
Morphology in fluidized bed reactors via aliquot on plates	Not given	Not given/internal scale	Not given	Image digitized in ScanJet IIcs (Hewlett Packard); computer program (Statgraphics software, STSC)	[63] (*Aspergillus, Phanerochaete*)
Morphology in broth samples diluted 20–100 ×	Camera; Optiphot microscope (Nikon)	60–200 × /internal object	Not given	Magiscan 2A image analyzer (Joyce Loebl)	[70] (*Penicillium*)
	Camera; Polyvar microscope (Leica)	400 × (hyphae)/ not given	Not given	Magiscan MD system (Joyce Loebl) running image analysis software	[71] (*Penicillium*)
Pellet broth samples diluted 70 × (pellets filled ≤ 10–15% of total area)	Computer-controlled microscope stage (Zeiss) with autofocus control	25 × /not given	Not given	TV monitor; image processing system (IPS, Kontron) with custom programs; pellets recognized based on prior interactive classification of numerous images	[85, 86] (*Streptomyces*)
Pellet diameter and number	CCD camera (MCC) attached to microscope (Leica)	Not given	Not given	Image analysis system (Leica)	[87] (*Aspergillus*)
Morphology in broth samples diluted 3,000–8,000 ×	Monochrome CCD camera (Sony); Axiovert microscope (Zeiss)	150 × /not given	Not given	TV monitor; IBAS image analyzer (Kontron); transformed into black and white image (binarization); low-pass filter used to obtain clear image for measurement	[102] (*Streptomyces*)
Broth sample removed (no dilution mentioned)	Microscopy using Thoma counting chamber	Not given	Not given	Digital image analysis software (KS300, Zeiss)	[32] (*Aspergillus*)
Broth sample removed and examined (no dilution mentioned)	CCD camera (Sony); Diaphot microscope (Nikon)	400 × /not given	Not given	Digitized using frame grabber (Data Translation); Image Pro Plus (Media Cybernetics) software; morphological parameters determined by custom program in commercial software package (Visilog)	[4] (*Methanosaeta*)

Table 2 continued

Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References (culture genus)
Fungal shape classification; diluted (not given) samples	Color slides (Kodak Ektachrome 160); Bosch video camera (Chalnicon tube)	Not given	Not given/400 ASA	Professional package (Visilog) or custom software	[79, 106] (*Penicillium*)
Anchorage-dependent cells on microcarriers					[80, 81] (Human kidney, CHO)
Bacteria cell count in microflow channel with dilution (not given)	CCD monochrome camera (DAGE-MTI); microscope (Olympus)	1,000 × /not given	Not given	Image processing system (Datacube and Vision Systems); object classification using back propagation of a neural network	[49] (*Bacillus*)
Pellets from diluted (not given) broth	RW-CCD camera (Sony); microscope (Leitz) or macro lens	Not given	Not given	Image processing system (Viscom); TopPic software customized for semi-automated processing; clumping pellets divided manually	[82] (*Penicillium*)
Morphology from broth pellet samples; diluted to ~ 0.4 g/L	Polyvar optical microscope (Reichert); color CCD camera(Sony)	40–100 × /not given	Not given	Microscope connected to Quantimet 570 image analyzer (Leica) using customized software	[41, 105] (*Penicillium, Streptomyces*)
Morphology in broth samples diluted 2–10 × to obtain 10–50 pellets/mL	Chalnicon tube type monochrome video camera (Hammamatsu) with zoom lens	10–26 × /not given	Not given	Morphopericolor (Matra) image analyzer connected to PC with custom programs	[24] (*Fomes*)
	CCD color camera (Sanyo)	6–7 × /not given	Not given	Quantimet 570 image analyzer (Leica)	[25] (*Schizophyllum*)
Morphology in broth samples sieved and washed to remove free mycelia	Video camera; microscope	25–50 × /not given	Not given	Software (CUE-2 Galai Productions)	[21] (*Aspergillus*)
Morphology in broth samples diluted 2 ×, then distributed, so pellets separated	CCD camera (Hitachi); illuminated acrylic plate	Not given	Not given	ImagePro (Media Cybernetics) supplemented by customized program	[28] (*Cyathus*)
Morphology in diluted broth samples	CCD camera (Sony); microscope (Zeiss)	Not given	Not given	Image process system for capture/analysis (ITEX-OFG/IPA, Image Technology) and analysis (IPS, Kontron)	[104] (*Streptomyces*)
Morphology in diluted broth samples	CCD camera (Sony); Optishot microscope (Nikon)	Not given	Not given	Image capture board (I-photo, Creative Art), image analysis (Advanced American Biotechnology)	[90] (*Aspergillus*)
Morphology in flow-through cell	CCD camera (Bischke); microscope (Reichert)	Not given/fixed chamber	Not given	Image analyzer (Quantimet 6005)	[96] (*Aspergillus*)
Broth samples diluted to < 1 g/L biomass	< 600 μm pellets: Polyvar CCD camera (Sony); microscope (Reichert) > 600 μm pellets: CCD-TV camera (Sanyo); macroviewer (Leica)	40–200 × /not given	Not given	Quantimet (Leica) image analysis system	[77, 78, 105] (*Penicillium, Streptomyces*)

Table 2 continued

Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References (culture genus)
Broth samples diluted 2,000 ×	Microscope (Olympus), CCD camera (Sony)	40 × /not given	Not given	ULT image analyzer (Graftek Imaging)	[14, 15] (*Streptomyces*)
Broth sample from oil, water, and air dispersion	CCD camera (Nikon); Optiphot microscope (Nikon) or stereomicroscope (Olympus)	20 × /not given	Not given	Image Pro image analysis software (Media Cybernetics)	[52] (*Trichoderma*)
Hyphal length in fungal colonies	Video camera; microscope	Not given/calibrated electronic mouse	Not given	Image processing system using Biocom software (Imagenia 2000)	[50] (*Aspergillus*)
Hyphal growth on-line via flow-through cell	Microscope (Reichert); CCD camera (Bischke)	4–10 × /not given	Not given	Quantimet image analyzer system (Leica) using EC video (Panasonic)	[16] (*Aspergillus*)
Mycelial growth in chamber mounted on scanning table	TV camera with Chalnicon tube (Bosch); microscope (Zeiss)	Not given	Not given	Image processing system (IPS, Kontron)	[114] (*Streptomyces*)
Digester sludge granules from samples spread over glass plate	Not applicable	2.5 × /micrometer	Not applicable	Images acquired directly with Epson scanner; analyzed with UTHSCSA Image Tool Program (Univ. Texas); overlapping granules excluded	[36] (NA)
Digester sludge granule samples	CCD video camera (Sony) with 25 mm TV lens	None/1.5 mm ball bearings	Lens set at maximum aperture/ not given	Vidas digital image processing unit (Kontron)	[23] (NA)
Dispersed sludge sample diluted to lowest amount for maximum percent object recognition	> 0.2 mm used stereomicroscope (Olympus); < 0.2 mm used Axioscop microscope (Zeiss); CCD gray scale video camera (Sony)	40 × (> 0.2 mm); 100 × (< 0.2 mm)/ micrometer	Not given	Digitized using frame grabber (Data Translation); Image Pro Plus (Media Cybernetics) software with customized separate Matlab programs for filaments (0.01–0.1 mm) and micro (0.1–1 mm) and macro aggregates (> 1 mm)	[5] (NA)
Bitumen-air floatation cell	Handycam video (Sony) with lenses to increase magnification	8–10 × /not given	Backward illumination/ not given	VHS recording; professional editing recorder (JVC) permitting frame by frame analysis; Snappy Video Snapshot digitized selected frames; SigmaScan Pro automated image analysis software	[56] (NA)
Yeast from sample (no dilution mentioned)	CCD camera (Sony); Diaphot microscope (Nikon)	400 × /not given	Not given	Frame grabber (DT3155, Data Translation); Matlab v.6.1 image processing (The Mathworks)	[17] (*Saccharomyces*)
Automatic real-time detection and tracking of moving organisms	Monochrome CCD camera (Phillips); Axioplan microscope (Zeiss)	Not given	Not given	Video signal input to image system board (Image Technology); real-time gray level difference between two successive frames	[33] (*Euglena*)

Table 2 continued

Application	Camera	Magnification/ calibration	Illumination/shutter or frame speed	Image measurement and analysis method	References (culture genus)
Particle liquid flows in pipe	CCD camera (Minton)	Not given	Diffused back-light/ 0.0005 s	Digitized in real time by PC with image grabber	[72] (NA)
In situ microscopy with sampling chamber	CCD camera attached to objective	100 ×/defined measurement chamber	LED/not given	Digitized using frame grabber, image analysis software (AdOculos)	[37] (CHO)
	Two CCD cameras with beam splitter	20 ×/defined measurement chamber	Direct light (non-pulsed)/ not given		[8] (*Saccharomyces*)
Cell number in undiluted broth via cell chamber and automatic sampling	CCD camera (Pulnix); microscope (Nikon)	400–1,000 ×/fixed counting chamber	Not given	Image processing system	[115] (*Saccharomyces*)
Optical density and cell count in situ	CCD camera with zoom and microscopic lens option	Not given	HYL80 fiber optic light (Canty)/0.00001–0.017 s	Vector System (Canty) for image analysis	Process Microscope Camera System, Canty Process Technology (http://www.JMCanty.com) (yeast)

Light source typically provided by microscope

expressions used to describe diameter. The sample (arithmetic) mean bubble diameter, d_a, is given by [6]:

$$d_a = \left(\sum d_i\right)/n, \tag{1}$$

where n is the total number of bubbles measured and d_i the diameter of bubble i. The Sauter mean diameter, d_{32} [9, 69, 97], links the area, $n_i d_i^2$, and volume, $n_i d_i^3$, of the dispersed phase (the number of bubbles, n_i, of diameter d_i), as shown by:

$$d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2. \tag{2}$$

This diameter is important for quantifying mass transfer effects [110].

The log-geometric mean diameter, d_g, is calculated using [75]:

$$d_g = \left(\sum n_i \log d_i\right) / \sum n_i. \tag{3}$$

It characterizes the log-normal distribution curve, one type of distribution commonly associated with bubble size distributions in agitated systems. Air–water bubble diameter distributions, plotted as the percent relative frequency versus the equivalent spherical bubble diameter, d, also fit the Weibull distribution. An exponential distribution was approximated for air–electrolyte bubble distributions in a 0.15 M NaCl solution [6, 30]. Thus, more than one distribution function may be used to fit bubble size distributions from gas–liquid dispersions.

Another diameter expression, the volumetric mean diameter, d_{30}, is obtained when the equivalent diameter is back-calculated from total volume measurements by assuming a spherical shape [59] according to:

$$d_{30} = \left(\sum 6V_b/\pi\right)^{1/3}/n, \tag{4}$$

where V_b is the total volume of bubbles. Volume also can be estimated from the cross-sectional area, A (assuming a spherical shape), where the circularity, C, shape, SF, or form factor [25, 56, 66, 89], a quantitative description of non-sphericity based on the perimeter, P, are given by:

$$\mathrm{SF} = 1/C = 4\pi A/(P^2), \tag{5}$$

where SF = 1 for a perfect circle and SF approaches 0 for a line [56]. The Feret diameter, d_F, or equivalent circular diameter [56], is the equivalent diameter of a circular object with the same area as the irregular object being measured according to:

Table 3 Summary of bubble measurement parameters from the literature in ascending order of objects per measurement

Measurement type	Measurement time per condition	Number of objects per measurement	Measurement error/ size range	Number of images (pictures or frames) per measurement	References
Interfacial area	Manual	Not given	± 5%/not given	Not given	[88]
Size distribution	Manual	50–100	10–15%/3–4 mm	2–3	[46]
d_{30}	Not given	100	Not given/0.2–1.0 cm	Not given	[59, 60]
Size distribution and velocity	Not given	200	Not given/2–8 mm	Up to 400	[91]
d_{32}/distribution	Not given	200	Not given/0.8–1.8 mm	Not given	[13]
d_{32}/distribution	Manual	> 300	< 15%/360–1,480 μm	Not given	[75]
Diameter distribution and position	Not given	480	< 15%/2.8 mm	3	[53, 94]
Sauter mean diameter[a]	3 min processing, 20 min from acquisition to printout	About 500	< 6%/0.4–5.0 mm	Not applicable	[6, 7, 30, 31, 99]
d_{32}	Not given	> 500	< 10%; 10 μm difference or < 2% std dev/500 and 1,200 μm	100–300	[52, 83]
d_{32}	30–60 min estimated (semi-automated, 1–8 min/frame)	> 500 > 800	10–20%/40–2,000 μm	4–25 (20–120 drops/frame)	[69] [68]
d_g and d_{32} distribution	Manual	> 500	4%/300 μm	Not given	[38]
Size distribution and mean diameter	Not given	> 500	Not given/40–5,000 μm, ≥ 20 μm	Not given	[27]
Size distribution and mean diameter	Manual	> 600 > 800	Not given/0.05–0.7 mm	Not given	[100, 101]
Bubble size distribution	Manual	350–1,100	5%/~ 0.5–7 mm	Not given	[95]
Bubble size distribution	Semi-automatic	800–1,000 (0.05–0.3 mm) 300–600 (1–20 mm)	Not given/0.05–20 mm	Not given	[26]
Bubble size distribution	Semi-automatic	800–1,500	Not given/0.25–1.5 mm	1,000	[10]
Size distribution and mean diameter	5 min	About 1,000	Not given/40–5,000 μm	10–100 (10–100 bubbles/picture)	[55]
Size distribution and mean diameter	10 min	1,000	Not given/≥ 0.4 mm	Not applicable	[9]
Size distribution	Not given	> 1,000	± 0.05 mm/≤ 3.5 mm	Not given	[92]
d_{32}	Manual	About 1,500	Not given/1.3–2.6 mm	Not given	[42]
Bubble size distribution	Not given	4,000	Not given/0.1–4 mm	500	[45]
Size distribution[a]	1–2.5 min (20–50 s^{-1})	About 3,000	2.822 ± 0.015 mm (n = 6)	Not given	[67]
Size distribution[a]	1.3 min (50 s^{-1})	Up to 4,000	Not given/0.75–3.5 mm	Not given	[84]

[a]Non-photographic methods

$$d_F = (4A/\pi)^{1/2}. \quad (6)$$

Other applicable shape descriptors [89] include the roundness, R, given by:

$$R = 4A/[\pi d_{long}] \quad (7)$$

and the aspect ratio, AR, given by:

$$AR = d_{long}/d_{short}, \quad (8)$$

where d_{long} and d_{short} are the longest and shortest diameters of the bubble, respectively. Quantification of non-sphericity assists in distinguishing between bubbles originating from spargers and those from other sources (such as vortex entrainment).

Table 4 Summary of pellet and morphological measurement parameters from the literature in ascending order of objects per measurement

Measurement type	Measurement time per condition	Number of objects per measurement	Measurement error/size range	Number of images (picture or frames) per measurement	References
Pellet core area	Not given	20	Not given	Not given	[14, 15]
Hyphal length	Manual	> 10–20	± 0.25 mm/200–300 μm	Not given	[57]
Hyphal length	Manual	30	Not given	Not given	[50]
Hyphal length	12 min	20–30	Not given/25–1,800 μm	5–10	[16]
Hyphal width	Not given	100	2–12%/5.2–6.8 μm	Not given	[71]
Pellet number and diameter	Not given	100–120	Not given/100–1,200 μm	Not given	[87]
Morphology (pellet diameter/hairy length)	Not given	> 150	Not given/0.2–3.8 mm (pellet core); 0.04–0.32 mm (hyphae)	Not given	[21]
Pellet morphology	Manual	100–250 (pellets), 50 (hyphae)	Not given (pellets), < 1–5% (hyphae)/100–800 μm	Not given	[12, 65, 66]
Hyphal width	1–2 h	150–250	2–3%/4.8 μm	64	[77, 78]
Pellet core area	5 min capture (1.3 s/pellet); 5 min analysis	150–400	< 3–5%/not given	20–30	[24, 25]
Hyphal width; free versus clumped mycelia	15–170 min (0.16 min/object)	100–200	± 20%/not given	15–200	[105]
Hyphal length and projected area	Not given	> 100 (length), > 250 (area)	Not given/140–220 μm	Not given	[41]
Pellet radius and distribution	Manual	About 200	Not given/0.05–0.2 mm	Not given	[103]
Mycelial particle size distribution	Not given	> 300	Not given/0.5–5 mm	Not given	[52]
Pellet diameter distribution	Not given	About 300	Not given/2.75–6.7 mm	Not given	[62, 63]
Granule size (diameter/area) and density	Not given	400	Not given/~ 1.5 mm	Not given	[23]
Cell velocity and direction	3.6–6 s; 0.3 s/frame	> 500 reconstructed object tracks	Not given	12 image time sequences	[33]
Hyphal length	~ 0.75–1.4 min/object (semi-automatic); 0.07–0.17 min/object (fully automatic)	About 100–1,000 (parameter dependent)	Not given/50–200 μm	10–200	[70]
Diameter distribution	Not given	About 1,500	± 10 μm/0.3–2.4 mm 0.007 pixels/mm^2 (area)	Not given	[36]
Pellet diameter distribution	Not given	About 2,000	Not given/1–6 mm	Not given	[28]
Cell area distribution and elongation	Not given	2,000 (elongation) 75–300 (area)	< 5% (elongation), not given (area)/not given	100 (elongation) Not given (area)	[17]
Pellets in diluted broth in measuring chamber	150 s/frame; few big separated pellets: ≤ 30 min; many filamentous mycelium: ≤ 3 h	300–5,600	5–17%/not given	28	[85, 86]
Aggregate size	15–40 s; 30 fps	Not given	< 27%/0.4–0.8 mm	3–12 (aggregate speed dependent)	[56]
Aggregation/filaments	Not given	Not given	Not given/0.0038– > 1 mm	100 (34 images/slide)	[4, 5]
Particle position in liquid flow	9.4 s/set; 25 fps	12,000–24,000; 50 per frame: 5.5 mm, 20–100 per frame: 3.1 mm	1–2 pixels (0.1–0.2 mm) for position/3.1–5.5 mm	236	[72]

Regardless of how the diameter is obtained (direct measurement or back-calculation from area or volume measurements assuming a circular or spherical shape), a ''higher moment'' approach can be used to obtain the exact description of the bubble size distribution curve [42] for any distribution type. The standard deviation from the arithmetic mean diameter, σ_a, may be calculated according to [42]:

$$\sigma_a = \left[\left\{\sum n_i(d_i - d_a)^2\right\}/n\right]^{1/2} \quad (9)$$

or [6, 7]

$$\sigma_a = \left[\left\{\sum (d_i - d_a)^2\right\}/\{n-1\}\right]^{1/2}. \quad (10)$$

From this quantity, the coefficient of variation, C_v [6, 7], may be obtained using:

$$C_v = \sigma_a/d_a, \quad (11)$$

where C_v is the distribution spread relative to its mean.

Two other useful descriptions of a distribution are effective in describing its difference from a normal distribution. The skewness, A_3, is the third moment about d_a, divided by σ_a^3 to make the measurements unitless [42] and is given by:

$$A_3 = \left\{\sum n_i(d_i - d_a)^3\right\}/\{\sigma_a^3 n\}. \quad (12)$$

The kurtosis, A_4, is the fourth moment about d_a divided by σ_a^4 to make the measurements unitless [42] according to:

$$A_4 = \left[\left\{\sum n_i(d_i - d_a)^4\right\}/\{\sigma_a^4 n\}\right] - 3, \quad (13)$$

where the kurtosis of the normal distribution is 3.

For the log-normal distribution, the standard deviation, σ_g, is given by [75]:

$$\sigma_g = \left[\left\{\sum n_i(\log d_i - \log d_g)^2\right\}/n\right]^{1/2} \quad (14)$$

and the characteristics of the log-normal distribution curve may be used to calculate d_{32} according to:

$$\log d_{32} = \log d_g + 5.7565 \log^2 \sigma_g. \quad (15)$$

In Eqs. 14 and 15, the logarithm is the common (base 10) logarithm.

Presentation and analysis of bubble size distribution data

There are several methods in the literature for presenting bubble size distribution data.

Common plots are (1) the percentage number frequency (either incremental or cumulative) or number probability density (Y axis) versus (2) the bubble diameter in a specified range [27] or versus the number of bubbles less than the stated bubble size (X axis) [59]. Selection of size ranges or ''bins'' directly affects the accuracy of the distribution's calculated parameters and obviously cannot be less than the established incremental size measurement range of the instrument. Previously, increments of 80 μm [75] up to 0.25 mm [6, 7] were used for bubble size data. Smaller bins result in a more accurate smoothing of bubble size histograms or ''stepped'' cumulative distribution curves into probability density functions or cumulative distribution functions, respectively. Specifically, cumulative bubble volume distributions have been smoothed by three passes through a triangular digital filter to remove data discontinuities [76].

Specific shapes of bubble size distribution plots can be expected. Plots of the normalized cumulative bubble volume distribution (Y axis) versus the log of bubble diameter (X axis) are sigmoidal in nature [83]. The log of the cumulative volume percent of bubbles of that diameter (Y axis) versus the log of bubble diameter (X axis) results in a nearly linear graph [29, 75]. Probability density versus bubble diameter results in a skewed distribution to the lower or upper bubble diameters depending upon the system [29].

Bubble distributions have been found to be non-normal [6], and specifically number frequency distributions were not symmetrical but showed positive skewness [101]. In some cases, size distributions, such as those obtained for bubbles from perforated plates, have been assumed to follow a logarithmic normal probability distribution [59]. Log-normal distributions, using geometrically increasing bin sizes to accommodate sizes ranging over a few orders of magnitude, have been used for agitated gas–liquid systems, along with calculating d_g as well as d_{32} [38].

Presentation and analysis of pellet size distribution data

Similar methods have been used to display pellet size distribution data with the exception that a somewhat broader range of possible quantities can be calculated. Key quantities are the percentage of pellets [109] or

the number frequency of pellets as function of size (e.g., radius) [86, 103]. Other obtainable quantities include the pellet concentration, pellet volume (sum of individual particle volumes), average diameter (e.g., d_a), volume concentration (volume of pellets per liter of sample volume), cumulative volume concentration curve (addition of individual volume concentrations from 0 to d_i), normalized cumulative volume concentration curve ($d_{max} = 1$), and median value of diameter, d_{50} (diameter for which normalized cumulative volume curve is 0.5) [28].

Pellet size distribution data have also been analyzed over the progression of a fermentation. The pellet fraction (pellet number of a certain size/total number) greater than a set size has been evaluated as a function of fermentation time [34], and the projected area of pellets has been explored for various bioreactor operating parameters [41]. A 3D graph has been constructed using the percentage pellet frequency (Y axis) as a function of fermentation time (X axis) and pellet radius (Z axis) [82]. The measurement procedure often is repeated for several samples, then the size distribution is calculated by averaging [82]. Furthermore, size distribution data have been linked with off-line data to plot the percent dry biomass versus pellet diameter [58] or to obtain the pellet density (division of dry cell weight by pellet volume), ρ_p [28].

Relationship of bubble size to operating parameters

A summary of the influence of operating parameters and liquid phase properties on bubble size in a gas/liquid dispersion is given in Table 5. Several theoretical and experimental relationships have been established to quantify these influences. These relationships are presented below, then used to quantify expected bubble size changes from expected operating parameter changes to estimate bubble measurement sensitivity requirements. In the future, a similar exercise can be conducted for pellet size changes, although the relationships are more complex owing to the varied nature of pellets relative to bubbles [48, 74, 107].

The interfacial area, a_{32}, is calculated according to [9, 97]:

$$a_{32} = 6\Phi/d_{32}, \tag{16}$$

where Φ is the void fraction of the dispersed phase or hold up. For typical Φ of 5–15% and d_{32} of 0.5–1.0 mm, a_{32} ranges from 0.3 to 3.0 mm^{-1}, and there are 50–150 bubbles/cm^3.

Many relationships have been established to relate bubble size distribution characteristics to operating parameters. Often experimental data are required to determine constants in these relationships [55]. Their accuracy depends on the precision of the bubble size measurement technique, and thus relationships can vary when measurement techniques differ among various researchers [55]. Similar statements apply to pellets relationships to operating parameters.

Both the maximum bubble diameter, d_{max}, and d_{32} correlate with the power input per unit mass, ε_T, surface tension, σ_T, and continuous phase density, ρ_c, according to [27, 101]:

$$d_{32} \text{ or } d_{max} \propto \varepsilon_T^{-0.4}\sigma_T^{0.6}\rho_c^{-0.6}, \tag{17}$$

where $d_{max} \sim d_{99}$, the diameter that is larger than 99% of all diameters in the cumulative number bubble distribution [75]. The proportional relationship between d_{32} and d_{max} was determined experimentally for bubbles produced by fine pore spargers [75]:

$$d_{32}/d_{max} = 0.63. \tag{18}$$

It was related to the parameters of the log-normal distribution by [75]:

$$d_{32}/d_{max} = \exp(2.5\ln^2\sigma_g - 2.33\ln\sigma_g). \tag{19}$$

A similar approach is expected to apply to bubble size distributions produced by open pipe or ring spargers.

Another established correlation is the Calderbank equation [55] for gas/liquid and liquid/liquid systems:

$$d_{32} = A_c[\sigma_T^{0.6}/\{(P/V_L)^{0.4}\rho_c^{0.2}\}]\Phi^{\beta}(\mu_G/\mu_L)^{0.25}, \tag{20}$$

where A_c and β are determined experimentally, P/V_L is the gassed power input per unit volume, and μ_G and μ_L are the viscosities of the gas (air) and liquid (water/electrolyte) phases, respectively. This equation has been simplified by various researchers:

Gas/liquid mixtures [55]

$$d_{32} \propto N^{-1.2}\sigma_T^{0.6}, \tag{20a}$$

Gas/liquid mixtures [111]

$$d \propto N^{-1.5}, \tag{20b}$$

Oil/liquid mixtures [111]

$$d \propto N^{-1.2}, \tag{20c}$$

Table 5 Factors affecting bubble size in stirred tank bioreactors

Parameter (increase)	Effect	Relative change for two-fold parameter increase	Probable cause	Equation/data	References
Power per unit volume, P/V_L	Decrease	− 0.76	Greater bubble breakup	$(P_g/V_L)^{-0.4}$	[55]
Impeller speed, N	Decrease	− 0.44		$N^{-1.2}$	[101, 111]
		− 0.35		$N^{-1.5}$	[55]
			Greater decrease at lower Q; increased uniformity of size distribution		[6, 7]
Volumetric gas flow rate, Q	Slight increase	+ 1.07		$Q^{0.1}$ (region of impeller)	[101]
		+ 1.15–2.0		Q^{γ}, where γ = 0.2–1.0	[108]
			Increased probability of coalescence due to higher bubble density; reduced turbulence level due to larger impeller gas cavities		[6, 30]
Superficial velocity	Slight increase	+ 1.11	Increased bubble collision frequency; higher coalescence rate	2.6 mm–0.39 cm/s 2.45 mm–0.25 cm/s	[92] (nitrogen)
Gas molecular weight (listed in parentheses)	Slight increase	+ 1.07	Decreased gas cavity length, lower surface area, less ability to form tiny bubbles	*0.690 mm: xenon (262)* 0.540 mm: air (29) 0.456 mm: helium (8)	[101]
	Slight decrease	− 0.99	Higher μ_G with higher molecular weight (similar effect as liquid viscosity)	0.446 cm: argon (80) 0.452 cm: air (29) *0.45 cm: nitrogen (28)*	[112]
Pressure	Increase	− 0.99	Reduced liquid phase turbulence (less bubble breakage)	1.1 mm: 46 bar 1.9 mm: 1 bar	[92]
Temperature	Decrease	− 0.92	Lower σ_T at higher temperature	2.62 mm: 40 °C 2.85 mm: 20 °C *2.96 mm: 10* °C	[67]
pH	Increase	+ 1.16		2.85 mm: pH 7 2.55 mm: pH 4	[67]
Ionic strength	Decrease	− 0.95		2.4 mm: 0.5 mol/L 2.7 mm: 0.16 mol/L	[67]
			Retarded bubble coalescence		[6]
Surface tension, σ_T	Large increase	Not practical to extrapolate	σ_T more important at low than high Q for low μ_L		[44]
			Surface-active agents decrease bubble coalescence	1,100 μm: 0 g/L BSA, 73 dyn/cm 550 μm: 0.02 g/L BSA, 57 dyn/cm 425 μ: 0.2 g/L BSA, 55 dyn/cm	[83]
		+ 1.52		$\sigma^{0.6}$	[55]
Viscosity, μ_L	Increase	+ 1.14	Slower liquid film formation rate traps more air in each bubble; lower σ_T at higher μ_L	3.0 mm: 1.25 cP 2.6 mm: 0.6 cP	[67]
			Reduced liquid phase turbulence (less bubble breakage)		[92]
		+ 1.07	Dampened turbulent eddies	$\mu_L^{0.1}$	[112]
Filamentous (more than pellet morphology)	Decrease		Segregation at high biomass concentrations with dispersed mycelia	3.0 g/L biomass: 300 μm—clumps and dispersed mycelia mixture; 470 μm—pellets 1.0 g/L biomass: 470 μm—both forms	[52]

Relative changes calculated from given equation or extrapolated/interpolated from data given. Data in italics not used

Gas/liquid mixtures [101]

$$d_{\max} \propto N^{-1.2}. \tag{20d}$$

Clearly diameters decrease with increasing impeller speed but not as much as implied by Kolomogoroff's theory of isotropic turbulence [101].

Increasing gas flow rates at constant agitation speed shift distributions towards slightly larger bubbles as bubble density increases with higher gas hold-up leading to greater bubble collision and coalescence rates [6, 30]. Increased superficial gas velocity increases bubble collision frequency leading to higher coalescence rates and greater stable bubble diameters in bubble columns [92]. The effect of gas flow rate on bubble size, for bubbles generated from an orifice, has been quantified by [61, 108]:

$$d \propto \mathrm{Q}^{\gamma}, \tag{21}$$

where γ = 0.2–1.0 for gas flow rates, Q, for Newtonian fluids.

The influence of impeller speed, N, on bubble size cannot be properly quantified without considering it together with Q, according to [101]:

$$d_{32} \propto N^{-0.50} Q^{0.10} \tag{22}$$

for the impeller region. Increasing N shifts distributions towards smaller bubbles particularly at lower Q; at higher Q, this effect is less pronounced as bubble coalescence is higher [6]. Increasing Q causes a reduction in the turbulence level as impeller gas cavities grow and velocity fluctuations are dampened [30].

Gas bubbles tend to become smaller with lower σ_T, higher ρ_G, greater gas molecular weight [101], and decreasing μ_L [92]. Bubble size distribution shifts to smaller sizes as protein concentration increases (resulting in higher volumetric gas–liquid mass transfer coefficients, $K_L a$) due to a drop in σ_T [83]. Bubble sizes observed with dispersed mycelia are smaller than those observed with pellets due to segregation occurring at biomass concentrations (> 1.5 g/L) for the dispersed mycelia [52].

For a floatation model system, higher pH results in increased bubble size and higher ionic strength decreases bubble size, but the effect is less than that of pH [67]. An ionic solution (0.15 M NaCl) retards bubble coalescence substantially which causes bubble sizes to drop for similar conditions [6]. Higher temperature results in lower σ_T and thus decreased bubble size [67].

Larger bubbles are produced at higher μ_L because as μ_L decreases possibly liquid films form faster and trap less air in each bubble [67]. The effect of μ_L on bubble sizes in gas/liquid mixtures is quantified by [111, 112]:

$$d \propto \mu_{\mathrm{L}}^{\delta}, \tag{23}$$

where δ is 0.1 for air–aqueous dispersions. Specifically, for a change in μ_L of 1 cP (water) to 6 cP (50 vol.% glycerol), bubble size is expected to increase 20%. The validity of the correlation needs to be considered relative to the manual size analysis conducted from photographs by these researchers based on available technology. In the case of non-Newtonian fluids where μ_L changes as a function of shear, a suitable equation for the apparent viscosity is necessary to relate it to operating parameters such as agitation speed [3, 43, 64].

Estimates of the relative impact of a twofold change in these parameters on bubble diameter are given in Table 5. The suitability of novel bubble size distribution measurement devices can be assessed by the instrument's ability to reproduce these trends both qualitatively and quantitatively. Specifically, requirements for accuracy and precision may be obtained from these relationships.

Summary

Past and current image analysis technologies for bubble and pellet size measurements have evolved based on available measurement, data acquisition, manipulation, and storage technologies. Substantially more effort has been placed on cell, pellet, and morphological measurements than on bubble measurements as off-line sampling errors were lower although not non-existent for cellular materials. New instrumentation technologies are desired to perform on-line, in situ measurements on a time scale relevant to analysis and control for PAT applications. Sensitivity of these techniques needs to be sufficiently high, and measurement variability sufficiently low, so that the expected effects on bubble size distribution caused by changes in process conditions and/or broth composition are clearly characterized.

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