Mar. Biotechnol. 1, 495–507, 1999

MARINE BIOTECHNOLOGY

Bioprocess Intensification for Production of Novel Marine Bacterial Antibiotics Through Bioreactor Operation and Design

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Abstract: There is a lack of research into bioreactor engineering and fermentation protocol design in the field of marine bacterial antibiotic production. Most production strategies are carried out at the shake-flask level and lack a mechanistic understanding of the antibiotic production process, offering poor prospects for successful scale-up. This review shows that data need to be collated on media and physical optima differences between the trophophase and idiophase, along with investigations into the control mechanisms for biosynthesis, to allow implementation of novel fermentation protocols. Immobilization may play a part in bioprocess intensification of marine bacterial antibiotic production, through again this area is understudied. Similarly, mass transfer and shear stress data of fermentations are needed to provide the bioreactor design requirements to intensify antibiotic biosynthesis, with process scale-up in mind. The application of bioprocess intensification methods to the production of antibiotics (and other metabolites) from marine microbes will become an important strategy for improving supply of natural products, in order to assess their suitability as chemotherapeutic drugs.

Key words: Natural products, antibiotics, marine bacteria, bioreactor, bioprocess intensification

Introduction

There is growing interest in marine biotechnology, especially novel marine natural products, stemming from the potential of the vast untapped resource of genetic diversity found in marine life to harbor new chemicals (Fenical, 1993; Jensen and Fenical, 1994). In particular, novel secondary metabolites, including antibiotics, from marine bacteria are attracting attention because of the growing demand for new antibiotics (Levy, 1998). Many unusual antibiotic types have been discovered (James et al., 1996; Yoshikawa et al., 1997), although more have not been structurally characterized (Lemos et al., 1986; Mearns-Spragg et al., 1998). There has been little concerted effort to move the biotechnological process forward beyond the characterization phase, although a number of researchers have touched on aspects dealing with such bioprocess intensification (Imada and Simidu, 1992; Chandrasekaran, 1996; Mearns-Spragg et al., 1998). Before a pharmaceutical company can invest in expensive drug development programs, a reliable supply of the compound must be available (Faulkner, 1993). One of the major barriers to this is the lack of mechanistic understanding of the parameters involved in

Received March 11, 1999; accepted May 4, 1999.

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antibiotic biosynthesis in many producing microorganisms. This is especially true of marine bacteria, exemplified by Fenical (1993), who recommended a 100-L fermentation of seawater bacteria to yield sufficient amounts of target compounds for analysis, owing to yields of less than 1 mg/L. At the initial laboratory bench level, such a fermentation would ideally be carried out on a smaller, more intensive (and probably more cost-effective) scale, hence the need for bioprocess intensification.

Bioprocess Intensification

Process intensification is commonly used in chemical engineering to efficiently obtain higher product yields, often centering on increased pressures, turbulence, and temperatures to influence mass heat transfer and reaction kinetics; however, bioreactors generally operate within the narrow physiologic limits of the biocatalyst (Chisti and Moo-Young, 1996). Thus, bioprocess intensification focuses on optimizing fermentation yields via media composition and feed strategies, dynamic control of physical conditions, induction, genetics, immobilization, and bioreactor engineering. Much of the work in antibiotic production has been carried out on nonmarine microbes, mainly fungi (Nagamune et al., 1988; Vanek et al., 1990; Zhang et al., 1996), which is in contrast to the small body of work published on marine microbes. This review highlights areas in which empirical and mechanistic knowledge of terrestrial antibiotic fermentations (both fungal and bacterial) could be applied to marine bacteria. Adaptation of this current knowledge to new marine biocatalysts, along with further innovation, will help realize the pharmaceutical potential of novel marine antibiotics.

Secondary Metabolism

A fitting description of secondary metabolism, of which most antibiotics are examples, was made by Turner in 1971: "Secondary metabolism involves mainly synthetic processes whose end products, the secondary metabolites, play no obvious role in the economy of the organism." The advantage of such a producing bacteria is still debated today. The production of secondary metabolites classically exhibits a two-stage process, with a trophophase (or growth phase) and an idiophase (or production phase), usually when growth has slowed or stopped (Bu'lock, 1961). The general aim of any bioreactor producing secondary metabolites is to maximize the biomass in a short trophophase, while ensuring the best conditions for high, sustained, iodophase production.

Control of Antibiotic Synthesis via Growth Media

Carbon

The choice of carbon source greatly influences secondary metabolism and therefore antibiotic production (see reviews by Martain and Demain, 1980; Doull and Vining, 1990; Spizec and Tichy, 1995). A quickly metabolized substrate such as glucose may often achieve maximum cell growth rates, but is known to inhibit the production of many secondary metabolites. This "catabolite repression" is thought to be due to intermediates generated from the rapid catabolism of glucose interfering with enzymes in the secondary metabolism process. Gallo and Katz (1972), for example, observed that the enzyme that catalyzes the formation of the phenoxazinone ring of actinomycin was inhibited by glucose.

Fast-growing cells generally have secondary metabolism "switched off" until their growth rate slows, via feedback inhibition. This can lead to a fully biphasic fermentation profile, with no production during growth, only during the stationary phase (Liao et al., 1995). Marine bacteria have been shown to exhibit similar properties (Riquelme et al., 1996). Okami et al. (1976) showed that for a marine *Streptomyces* it was necessary to dilute the yeast extract in the growth medium (from a level found in traditional *Streptomyces* fermentations) to give quantifiable antibiotic production. Similarly, Okami et al. (1980) found that a marine *Bacillus* only produced a target enzyme on diluted nutrient media, suggesting that these are examples of facultative oligophiles. Slowly utilized substrates such as galactose (Basak and Majumdar, 1973) have improved antibiotic yields from fungi, exhibiting a less well-defined separation of trophophases and iodophases. The production of bacitracin by *Bacillus lichenformis* can be seen during the growth phase, when a slow growth medium is used (Haavik, 1974). The study of carbon source effects has not been comprehensively evaluated in regard to marine bacteria.

The examples cited highlight a dilemma between

achieving maximal growth rate and antibiotic yields (Chisti and Moo-Young, 1991), but innovative feeding regimens may overcome this. This was illustrated by Kaiser et al. (1994), who found that the total yield of manamycin from a *Streptomyces* was increased if glycerol was fed during the production phase.

Nitrogen

In a similar way to carbon, the nitrogen source is understood to regulate secondary metabolism. High nitrogen levels have been noted to repress iodophase production of antibiotics (for example, see reviews by Ahronowitz, 1980; Martain and Demain, 1980; Doull and Vining, 1990; Spizek and Tichy, 1995). Actinomycete isopenicillin synthase was found to be sensitive to ammonium levels (Demain, 1986). However, Zhang et al. (1996) found ammonium to stimulate an antibiotic produced by *Streptomyces griseofuocus.* Control of ammonia concentration during the mid-cycle was found to be important in the optimization of idiophase secondary metabolite production (Junker et al., 1998), though this may reflect the role of nitrogen in growth promotion.

The use of unsuitable amino acids as a nitrogen source can inhibit good synthesis of secondary metabolites (Ahronowitz, 1980; Martain and Demain, 1980). Conversely, specific amino acids can, in some cases, enhance antibiotic production. The addition of cystine, for example, increases the phenazine-1-carboxylic acid yield in *Pseudomonas fluorescens* (Slininger and Jackson, 1992), and methionene is well known for its stimulatory effect on cephalosporin C biosynthesis, when added during the growth phase of *Cephalosporium acremonium* (Martain and Demain, 1980). These effects may be due to a specific amino acid interacting with the regulation of secondary metabolic pathways. Again, as for carbon, little is known about the effect of amino acid concentration or type on antibiotic synthesis in marine bacteria. Multi-input feed strategies may be of use in process intensification, after optimization of the nitrogen type and concentration at different fermentation stages.

Phosphate

Phosphate, although essential for growth, can at certain concentrations suppress secondary metabolism, inhibiting, for example, phosphatases and oxygenases (Spizek and Tichy, 1995). Interestingly, the level of adenosine triphosphate (ATP) has been observed to decrease significantly before secondary metabolism begins (Cardova et al., 1976). This was attributed to the rise in phosphatase activity after the cessation of growth. Inorganic phosphate represses the synthesis of phosphatase, allowing the ATP level to remain high, which Behal et al. (1979) credit for repressing secondary metabolism. The restimulation of growth phase, if phosphate is added, may give rise to feedback inhibition of secondary metabolism.

Phosphate limitation was instrumental in stimulating phenazine-1-carboxylic acid synthesis by *P. aurofaciens* (Turner and Messenger, 1986) and phystigimine production by *Streptomyces griseofuscus* (Zhang et al., 1996). Phenazine production by *P. phenazinium* was found not to be regulated by phosphate level (Messenger and Turner, 1983), highlighting the fact that regulation of secondary metabolism can be species specific. This poses problems in applying general fermentation principles to such a diverse group of bacteria as those found in the marine environment.

Trace Elements

The final consideration in terms of basic media composition for secondary metabolite production is which trace elements to add (Weinberg, 1970). In terms of marine bacteria, the concentration of bromide ions would seem to be significant in some cases, probably because of the prevalence of bromide in halogenated marine antibiotics (van-Pee, 1996). One marine antibiotic obtained from a pseudomonad contained five bromine atoms per molecule (Burkholder et al., 1966), and medium with added bromide ions was found to give increased antibiotic production, over normal medium (Gauthier and Flatau, 1976; Ivanova et al., 1998).

The effect of specific metal ions on *Burkholderia glumae,* with regard to secondary metabolite production, was investigated by Yamaski et al. (1998), who concluded that certain ions gave optimum yields, whereas if $MgCl_2$ was replaced by $MgSO_4$, no production occurred. Zinc sulfate was found to increase phenazine production by *P. fluorescens* (Slininger and Jackson, 1992), similarly KNO_3 or $FeCl_3$ increased phytotoxin produced by *P. syringaei* (Palmer and Bender, 1993). This has interesting implications for the control of antibiotic production, if similar mechanisms are observed in marine bacteria.

Control of Secondary Metabolism by Altering Physical Parameters

Temperature

In common with media considerations, physical factors can exert differing effects on the growth and production phases of secondary metabolism. For example, marine *Alteromonas* grew best at 28°C but yielded more antiviral compound at 25°C (Myogga et al., 1995). Temperature is a regulatory factor in the secondary metabolism of *Streptomyces thermaviolaceus* (James et al., 1991) and, when reduced from its natural level, increased mycotoxin production from a wild marine *Aspergillus* (Tepsic et al., 1997). Consequently, temperature shifts or cycles may be of use during fermentation. This is illustrated by a lactobacillus, which increased the synthesis of an exopolysaccharide when the temperature was shifted from 37°C to 25°C at the beginning of the exponential phase (Gamar Nourani et al., 1998).

pH

The pH level of the growth medium has a marked effect on secondary metabolite production, with synthesis falling rapidly either side of an optimal level. This was the case for violacin production from the marine bacterium *Alteromonas leuteoviolacea*, which fell to 0 at pH 9 after an optimum had been reached at pH 7 (McCarthy et al., 1985). Similarly, *P. fluorescens* phenazine production declined rapidly at pH 8, after an optimum at pH 7 (Slininger and Shea-Willbur, 1995). Hays et al. (1997) used pH as a stressor to induce methylomycin synthesis by *Streptomyces* sp., a technique that merits further investigation as a bioprocess intensification tool.

Oxygen

The oxygenation of cultures is widely recognized as critical for optimal growth in aerobic fermentations. The increase of partial pressure O_2 was found to induce new secondary metabolite synthesis by *Streptomyces parvulus* (Kaiser et al., 1994); conversely, O_2 limitation initiated secondary metabolism in *Sacchoropolyspora erytherea* (Clark et al., 1995). Friebel and Demain (1977a) found that oxygen caused the inactivation of gramicidin synthase, reducing gramicidin production by *Bacillus brevis.* This was overcome by sparging with nitrogen during the iodophase (Friebel and Demain, 1977b), further illustrating the advantages of dynamic control of fermentation conditions. The oxygen transfer from sparged air to the bacterial cell is partially dependent on the media composition (for example, see Svitel and Sturdik, 1995), viscous media being harder to oxygenate. Oxygen transfer ought to be one of the first parameters optimized in reactor design, as this is commonly a major limiting factor.

Salinity

Some bacteria (though not all) living in the marine environment possess a salt requirement for growth (Kogure, 1998), though the effect of salinity on secondary metabolism has not been extensively researched. Salinity was found by Okami et al. (1976) to effect the production of aplasmomycin from a marine *Streptomyces.* Obviously, optimum salinity levels must be established for both growth and production phases. Reducing levels from full seawater (about 34 parts per thousand) has been shown to increase growth, probably through decreasing the energy channeled to cytoplasmic salt regulation. High salt levels may cause problems with bioreactor corrosion, and they inhibit the dissolution of sparged oxygen into a water-based medium (Garcia and Gordon, 1992).

Pressure

It is not unreasonable to expect even intertidal microbes to be tolerant of pressure fluctuations (probably between atmospheric pressure to nearly double that (10 m water depth = 202.6 kPa) at high tide). Indeed, bacteria associated with surface film seem to have a higher barotolerance than those not associated with surface film, so they may be able to withstand greater pressures (Wright et al., 1999). The pressure tolerance could be harnessed in a hydrostatic pressure bioreactor to improve O_2 transfer (Zobell and Hittle, 1967) via higher oxygen partial pressure (Enns et al., 1965) without increasing shear stresses. Hydrostatic pressurization to relatively low pressures can be achieved with only minor alterations to a "standard" bioreactor, but can markedly improve parameters such as maximum cell dry weight (Yang and Wang, 1992). Furthermore, the effect of pressure may act as a stressor to increase metabolite production via Le Châtelier's principle, if a negative volume change in reactants to products is favored (Wright et al., 1999). The effect of small pressure increases on secondary metabolism of nonbarophillic bacteria has not been investigated, with most biotechnology work concentrating on barophiles (e.g., Nelson et al., 1992).

Other Factors Affecting Regulation of Antibiotic Production

Induction

So far most, if not all, of the factors reviewed can have differing effects on secondary metabolite biosynthesis and cell growth. If one could somehow induce antibiotic production during the growth phase, overriding feedback inhibition, then antibiotic yields would most likely increase. This has already been achieved in *Streptomyces* culture, where if virginiae butanolides (an autoregulator for antibiotic synthesis) was added after 8 hours, virginomycin production doubled (Young et al., 1995). Another *Streptomyces* regulator, the "A" factor, is thought to bind to a special protein repressor, which, if left unbound, acts as a repressor of physiologic differentiation (linked with secondary metabolism) (Honouchi and Beppu, 1994). Similarly, the σ factor has been found to be necessary for stationary phase production of antibiotic by *P. fluorescens* (Sarniguet et al., 1995).

Homoserine lactones, the bacterial signal molecules equivalent to pheromones, are known to stimulate biosynthesis of a carbapenem antibiotic in *Erwinia carotovora* (Salmond et al., 1995), and were found to induce phenazine antibiotic biosynthesis in *P. aeruginosa* by Stead et al. (1996). Bassler et al. (1997) reported the cross-species induction of luminescence using homoserine lactones, opening possibilities for further control over secondary metabolite production in a bioreactor system, in which the production phase could be initiated earlier during fermentation.

Another method of inducing antibiotic production is by antagonizing the bacteria into defending itself against a perceived threat, via the production of antibiotic. Fredrickson and Stephanopolous (1981) stated that microbes competing for a single nutrient would try to eliminate one another via toxic warfare. Lemos et al. (1991) found that in mixed culture antibiotic-producing marine bacteria dominated nonproducers. Furthermore, Gil-Turnes et al. (1989) found that a marine *Alteromonas* produced an antibiotic to inhibit a potential fungal competitor. This theory has been developed into a method for inducing enhanced antimicrobial compound production by marine bacteria, by using terrestrial bacteria as antagonists (Mearns-Spragg et al. 1998; Burgess et al., 1999).

Induction does not necessarily have to utilize microbial products; the antibiotic production of a marine *Bacillus* was found to be dependent on the addition of amino acid analogue, selenomethionine (Imada et al., 1998). Such control of secondary metabolism so as to override normal primary metabolic repression has much scope in the intensified production of antibiotics.

Inhibition

Autoinhibition of antibiotic-producing microbes is well documented in marine bacteria (Anderson et al., 1974; Lemos et al., 1986), in which the concentration of the product negatively regulates its biosynthesis and could hamper process intensification efforts. The use of a continuous or semicontinuous fermentation protocol would allow extraction of any inhibitory compounds from the media, ensuring no repression of antibiotic production rates.

Genetics

Genetic manipulation has intensified antibiotic production in terrestrial microbes (for example, see Hosoya et al., 1998). However, no studies have been carried out on enhancing antibiotic production from marine bacteria, though some work has been carried out on marine bacteria for other applications (Angles et al., 1993; Tsyjibo et al., 1996; Lloyd et al., 1997). Genetic engineering in cyanobacteria is well established (for example, see Matsunaga and Takeyama, 1995).

Immobilization

Many different advantages have been assigned to live cell immobilization, though relatively few have been rigorously tested or gained acceptance in industry. Dervakos and Webb (1991) thoroughly review the advantages that have been associated with immobilization of viable cells, as summarized in Table 1. The major disadvantages associated with immobilization are also highlighted in Table 2.

The actual method of immobilization depends, of course, on the individual characteristics of the bacteria (Karel et al., 1985); but interestingly a recent paper by Ivanova et al. (1998) stated that the antibiotic production of 12 strains of epibiotic marine bacteria was enhanced after immobilization onto a polymeric surface. They found that hydrophobicity (measured via liquid-solid contact angles) played an important role in inducing immobilization, showing that hydrophobic surfaces had a greater number of attached cells. Conversely, the work of Bright and Fletcher

Table 1. The Advantages Conferred by Immobilization of Live Cells over Suspended Culture

Advantage	Example
Enhanced biological stability	Can tolerate stresses better (Karamanev and Nikolov, 1991)
High biomass concentrations	Little loss of cells through washout (Godia et al., 1987)
Improved mass transfer	Reduced media viscosity, increased differential velocity between media and cells (Wang et al., 1984)
Advantageous partitioning effects	Support can decrease inhibitory product concentration near cells (Wada et al., 1981)
Increased product yield	Energy is channelled away from biomass synthesis, into product synthesis (Sayles and Ollis, 1989)
Increased product stability	Liquid throughput reduces a product's residence time and therefore possibility of degradation (Okada et al., 1987)
Integration with downstream processing	Cells easily separated from media and products (Furusaki, 1988)
Increased reaction selectivity	Immobilization can affect membrane permeability (Watanabae et al., 1988)
Flexibility in terms of fermentation protocol	Step changes could be carried out with no deleterious effect of dilution (Godia et al., 1987)
Versatility of reactor selection	Can vary between plug flow to mixed tank; reactor less likely to clog (Wright and Raper, 1996)

Modified from Dervakos and Webb, 1991.

Table 2. The Major Potential Disadvantages Associated with Immobilization

Disadvantage	Example
Cell overgrowth	Overgrowth modifies reactor flow and affects mass transfer (Truck et al., 1990)
Support break-up	The support may possess poor mechanical stability (Kloosterman and Lilly, 1985)
Shear stress	Shear stress is higher than suspended cells owing to greater velocities (Fan, 1989)
Diffusion limitation	Substrate limitation/product inhibition in entrapped immobilization systems (Bailey and Ollis, 1986)

(1983) found that marine bacteria preferred hydrophilic substrata. CatalanSakairi et al. (1997) found that a polyester carrier was more effective than cellulose in a marine bacterial nitrification fermentation. Other influencing factors, such as pH, salinity and temperature, remain to be quantified; however, Costerton et al. (1995) have observed that *Pseudomonas aeruginosa* creates biofilms during periods of favorable nutrient conditions. Similar conditions (yeast extract and trypticose) have been used to speed reactor start-up (Yang et al., 1994), as the development of a mature biofilm is essential for an efficient process in any biofilm reactor (Annachhatre and Bhamidimarri, 1992).

Novel engineered support materials are being developed to improve immobilized bioprocesses, such as a nutrient-containing "plastic composite support," which outperforms normal polypropylene supports in ethanol production (Demirci et al., 1997). Another new support constructed from metal mesh particles aims to reduce the high shear stress sometimes suffered by immobilized bacteria (Kargi and Toprak, 1994). Successful immobilization would mean that semi or continuous production could be attempted and one could avoid cumbersome antibiotic bioreactor systems such as the multiple-tank setup of Reusser (1961).

Bioreactor Considerations

Reactor engineering determines parameters essential to bioprocess intensification—namely, oxygen and bulk media mass transfer, mixing patterns, shear stresses, and implementation of innovative fermentation strategies. Basic data regarding oxygen transfer requirements, mass transfer limitations, and mixing in bioreactors designed or operated for marine bacterial antibiotic production is absent from the published literature (for an exception dealing with a recombinant marine *Vibrio* producing a toxoid, see Lloyd et al., 1997). This contrasts to the design of marine photobioreactors, which have been the subject of extensive and innovative work (for example, see Contreras et al., 1998; Hu et al., 1998; Matsunaga and Burgess, 1994). Many marine bac-

teria are adapted to specific ecologic niches that impact on their physiologic behavior such as symbionts, epibionts, barophiles, psycrophiles, thermophiles, and oligophiles, the requirements of which can be incorporated in bioreactor design for improved process effectiveness. For example, cultivation of sponge symbionts may require culture of the sponge itself, to avoid the pitfalls of wild harvest (Osinga et al., 1998), or high-pressure reactors for barophiles (Yayanos, 1995). Most fermentations reported in the literature take place in shake flasks, which are likely to offer scope for bioprocess intensification, even on a small scale. This is illustrated by Wakisaka et al. (1998), who used a novel membrane reactor (growth on air-exposed surface of media-filled porous cylinder) to nearly treble the secondary metabolite production by *Aspergillus oryzae*, compared with shake flask cultures.

It is important to take account of the whole bioprocess; thus, consideration must be given to the type of downstream separation employed to obtain the secondary metabolite from a fermentation broth. Therefore, bioreactors need to be set up to allow ease of integration with downstream separation and purification strategies.

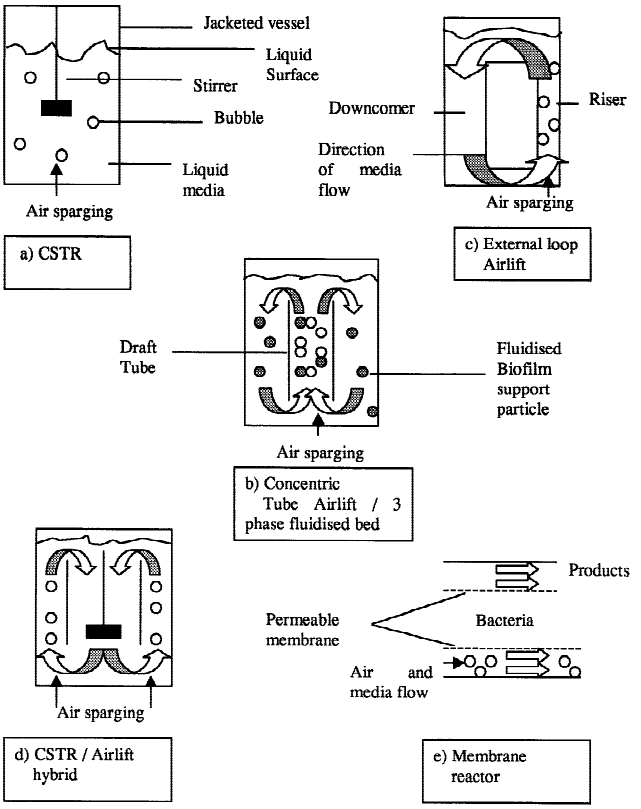

Figure 1. A schematic representation of different bioreactor configurations. Note that a three-phase fluidized bed configuration **(b)** can be implemented on all the reactors depicted, except **(e).**

Bioreactor Configurations

Five types of bioreactors will be discussed in proceeding sections, the most commonly used type of which is the continuous stirred tank reactor (CSTR, Figure 1a), which operates by mechanically mixing the air-sparged liquid medium in a jacketed vessel for temperature control. Another common reactor configuration is the airlift, which pneumatically mixes the medium, shown in concentric tube and external loop forms (Figures 1b and 1c, respectively). A hybridization of the airlift and CSTR can be seen in Figure 1d, utilizing both pneumatic and mechanical agitation. The final type of reactor that will be dealt with is the membrane reactor, consisting of two selectively permeable membranes that deliver nutrients and export products from the bacteria in the central tube (Figure 1e). Reactor types a to d (Figure 1) can be operated in a variety of modes, ranging from batch to continuous. A comparison of the relative advantages or disadvantages of CSTRs, airlift, and membrane reactors is shown in Table 3.

Increasing Oxygen-Media Mass Transfer

In many aerobic fermentations, one of the barriers to enhanced bioprocess effectiveness is oxygen transfer limitations. This has led to a number of modifications in reactor design such as the addition of a static mixer in an external-loop airlift (Figure 1c) to break up the sparged gas bubbles, thereby increasing the surface area for dissolution, raising the volumetric gas-liquid oxygen transfer coefficient (k_La) (Chisti et al., 1990). Again incorporating a mixer, but this time in a concentric tube airlift (Figure 1b), Pollard et al. (1997) found that the reactor showed more homogeneous dissolved oxygen levels than would otherwise be expected. To achieve better mass transfer characteristics without utilizing a mechanical agitator, Ghosh et al. (1993) modified the riser section in another external loop airlift (Figure 1c) reactor by using converging-diverging sections in the riser to increase turbulence.

Flat membrane reactors (Figure 1e) have improved their mass transfer characteristics when the membrane is dimpled, stimulating vortex mixing (Beeton et al., 1991). Indeed this reactor was successfully used to grow a pseudomonad to produce a phenazine antibiotic (Beeton et al., 1994).

Table 3. The Major Advantages and Disadvantages That Have Been Assigned to CSTRs, Airlift, and Membrane Reactors

Reactor type	Advantages	Disadvantages
CSTR	Nearly perfectly mixed Good oxygen transfer Extensively modeled	High shear stress High power to volume input Moving internal parts
Airlift reactor	Lower shear stress than CSTR Lower energy input than CSTR Can have no internal parts Easier to model than a bubble column	High viscosity can limit bulk circulation
Membrane reactor	Low shear stress Low downstream contamination High oxygenation rates	Biofilm overgrowth Membrane can rupture at high cell densities/ flow rates (if a fiber bioreactor)

Reduced Shear Stress and Mixing

An increase in the mixing velocity within a bioreactor can improve mass transfer; however, biocatalysts are often sensitive to shear fields caused by increased agitator speeds, leading to a drop in productivity. For example, the specific production rate of a penicillin fermentation (gram per cell dry weight) was over 50% less in a 6-L CSTR when the rushton turbine was operated at 1000 rpm compared with 600 rpm (Justen et al., 1998). Stirred tank reactors (Figure 1a) have benefited from different agitator types to decrease shear stress, and improve mixing (for example, see Oh et al., 1997; Wang and Zhong, 1996). CSTRs are still the most widely used and researched fermentation reactors, as they have high oxygen transfer characteristics (essential in oxygen-limited fermentations) and are relatively easy to model, even though they suffer, for example, from higher rates of shear stress compared with airlifts. A compromise between both designs is the hybrid stirred tank airlift reactor (Figure 1d), which achieves good bulk mixing at low shear stresses (Moo-Young and Chisti, 1988).

In an effort to reduce shear stress in an external-loop airlift reactor, Guo et al. (1997) placed the sparger high in the riser section (Figure 1c). Increasing the aspect ratio of concentric tube airlifts can lead to a greater ability to suspend solids (Ganzeveld et al. 1995), which is critical in a fluidized bed configuration, using immobilized biocatalysts on a solid support.

Scale-up

To increase antibiotic output it will be desirable to carry out fermentations on a larger scale. Predicting the effect of scale-up is simple if all parameters affecting the bacteria remain the same, or if their response to varying process parameters can be accurately predicted. Unfortunately, both scenarios are rarely encountered, and the fact that few marine bacteria have been characterized in terms of their physiologic response to process variables makes scale-up difficult to model effectively. Numerous empirical and semi-empirical scale-up relationships have been used to correlate variables such as oxygen mass transfer and shear fields or rates, with physical parameters such as agitation speed and reactor dimensions, equations for which can be found elsewhere (e.g., Bailey and Ollis, 1986; Shuler and Kargi, 1992). There is no published work on these standard scale-up procedures being applied in marine bacterial antibiotic systems. It would seem that scale-up in such a system would remain highly empirical, until a mechanistic understanding of the physiologic responses of the bacteria to process variables is achieved.

Power Consumption

Bioprocess intensification can be measured via the production of product per unit cost of fermentation, of which the cost of power input can be large. Gravilescu and Roman (1998) showed that airlift reactors consistently had higher energy efficiencies of between 30% and 40% in a number of antibiotic fermentations, when compared with stirred tank reactors. This reflects a general trend of airlift reactors being more energy efficient than stirred tanks (Chisti, 1989). Indeed, airlifts can be more efficient than bubble columns, as illustrated in Table 4, which shows that with 40% more energy input, the airlift yields a 150% increase in k_La.

Conclusions

Bioprocess intensification, though developed in some standard nonmarine fermentations, is in its infancy with regard to marine bacterial antibiotic production systems, which lack a mechanistic understanding of the relation between

Table 4. A Comparison of Energy Input and Oxygenation for Airlift and Bubble Column Bioreactor Types at Maximum Oxygen Transfer Rates

Reactor	Energy input (kWm^{-3})	k_La (h^{-1})
Bubble column	2.5	140
Air lift	3.5	350

Adapted from Sittig, 1982.

process variables (e.g., shear stress and oxygen transfer) and physiological response. Needham et al. (1992, 1994) attempt to elucidate the biosynthetic pathways of two marine bacterial antibiotics, but there are few other published studies in this area. Thus, most work carried out on such systems remains empirical. The realization of such mechanistic understanding requires a vast amount of research, which may prove difficult when dealing with such a diverse group of bacteria as those found in the marine environment. One hopes that common fermentation principles can be developed for bacteria isolated from similar ecological niches, leading to the design of an intensification process.

Media and physical factors must be optimized for both growth and production phases, to allow a dynamic control strategy to be implemented. Furthermore, the development of induction strategies and immobilization may act as process intensification tools. Bioreactor design governs the ability to intensify the bioprocess, the design requirements of which are almost unknown for marine bacteria.

To realize its full potential, microbiologists and chemical engineers need to combine to form an interdisciplinary approach, to derive reactors and scale-up criteria that can move the biosynthesis of novel marine bacterial antibiotics beyond the crude shake-flask level.

Acknowledgments

Jamie Marwick would like to thank the Engineering and Physical Sciences Research Council (EPSRC) for provision of a studentship (award 98315485). Phillip Wright and Grant Burgess acknowledge the Scottish Hospital Endowments Research Trust (SHERT) for the provision of a research grant (RG 9/99). Grant Burgess also acknowledges the British Council Tokyo.

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