Evaluation of polyurethane foam materials as air filters against fungal contamination

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ABSTRACT

Current air filters used in food processing or storage facilities are expensive and disposable. The ability to use polyurethane foam as air filters against fungal spores would be beneficial as they are both cheap and re-usable. The aim of this study was to evaluate the air filtration capabilities, in terms of fungal spores, of a selection of polyurethane foam(s) of differing combinations of pores per inch (PPI) (50 and 90 PPI) and thickness (15 and 20 mm). Environmental air was used as a source of fungal spores and membrane filtration was used to assess the filtration capabilities of the foams. Spores capable of passing through the foams were captured on cellulose nitrate membrane filters and quantified in CFU counts. Apart from the 50 PPI foam of 15 mm thickness, all the foam samples were effective at significantly reducing the number of spores. The PPI was found to be 2 times more influential on the efficiency of the foam material than the foam thickness. This may be explained through the higher number of pores present and the decrease in thickness of the ribs composing the microstructure of the foam as shown through scanning electron microscope (SEM) micrographs. These studies show that reticulated polyurethane foams at the selected PPI and thickness can be used as effective air filters against fungal spores.

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1. Introduction

Airborne particles of biological origins, known as bioaerosols, may include, amongst other living material, fungi and fungal entities (Yoon et al., 2008). Highly-resistant fungal spores are found in all environments, including air inside buildings and facilities. This characteristic of fungal spores would not be an issue were it not for the fact that fungi are capable of causing seriously adverse effects; they can damage buildings (May, 2001; Miller, Rand, & Jarvis, 2003), they can cause spoilage of certain foodstuffs in food storage facilities and they may cause allergic diseases of the airways in individuals who inhale the spores (Portnoy, Barnes, & Kennedy, 2004; Zukiewicz-Sobczak et al., 2013). There are several ways by which bioaerosols can be controlled. These include ultraviolet germicidal irradiation (UVGI), air ionisation, dielectric barrier discharge and others (Griffiths, Bennett, Speight, & Parks, 2005; Jankowska, Reponen, Willeke, Grinshpun, & Choi, 2005; Ko, First, & Burge, 2002; Park, Yoon, Kim, Byeon, & Hwang, 2009; Park & Hwang, 2013; Schmid et al., 2013; Woo et al., 2012). The most commonly used means of eliminating bioaerosols from the internal environment of facilities is by air filtration (Brincat et al., 2016).

Air filtration systems are important for different types of protected environments, such as medical and food processing facilities, and less commonly in food storage environments. The most common air filtration systems to reduce the abundance of fungal spores in protected environments (especially in medical wards) are High Efficiency Particulate Air (HEPA) filters. HEPA filters have been shown to reduce the incidence of invasive aspergillosis (Abdul Salam, Karlin, Ling, & Yang, 2010; Alberti et al., 2001; Araujo et al., 2008; Vonberg & Gatsmeier, 2006). Studies have shown that fungal spores are much more abundant in environments that lack an air filtration system (50–500 CFU per m$^{-3}$) than in those which have functioning and effective air filtration systems (0–50 CFU per m$^{-3}$) (Brenier-Pinchart et al., 2009; Dassonville...
et al., 2008; Sautour et al., 2009). In order for this to be true, air filtration systems must be appropriately maintained and replaced when necessary so as to ensure the utmost quality of filtration. In fact, it is not uncommon for outbreaks (most commonly involving Aspergillus fumigatus) to occur due to the negligence of faults and contaminated air filtration systems (Lutz, jin, Rinaldi, Wickes, & Huycke, 2003; Munoz et al., 2004; Reboux et al., 2014).

The importance of optimally functioning air filtration systems is not restricted to medical environments. The quality and quantity of air in food manufacturing and storage facilities is regulated so as to prevent or significantly reduce the growth of fungal organisms which would otherwise cause severe spoilage of food stuffs, particularly grains, flours, nuts, ripening cheeses and fruits (Isaac, 1996). The primary concern of fungal contamination of food stuffs is the generation of mycotoxins, which are toxins generated by toxigenic fungal organisms within certain temperature and relative humidity ranges. Fungal species commonly of interest in terms of food contamination include those belonging to the Alternaria, Claviceps, Fusarium, Penicillium and Stachybotrys genera (Milicic, Skrinjar, & Baltic, 2010). The durability and heat resistance of these toxins makes them particularly difficult to eliminate as they are not destroyed by common cooking procedures such as frying and boiling (Magan & Olsen, 2004; Miličević et al., 2010).

When consumed, food stuffs contaminated with mycotoxins can cause a variety of acute and chronic health disorders in both humans and animals alike (Miličević et al., 2010). As a result, considerable attempts are made to prevent the contamination of food stuffs by fungal organisms (Magan & Olsen, 2004).

The regulation of air is most commonly brought about by filtration, which eliminates potentially harmful micro-organisms from the air that is being introduced into the facility by capturing and retaining them in the filter medium (EHEDG, 2006). Fungal spores may enter food production and storage facilities via drains, doorways, hatches, disinfection tunnels, compressed air supplies, raw materials, packaging, people handling and poorly designed, cleaned and maintained air filtration systems, amongst others. The latter source of airborne contamination is of particular interest. Functioning air filtration systems are designed to distribute fully conditioned air as required at the desired rate. The air handling systems must be able to not only remove contaminating micro-organisms from the air by filtration, but also to reduce or prevent growth of micro-organisms, prevent their ingress, curtail cross-contamination, direct bioaerosols away from the food stuffs and not act as an additional source of contamination (EHEDG, 2006). Systems must be designed in such a way to allow maintenance to be performed on the filter media. Lack of proper maintenance might lead to the filter itself becoming a source of spores in the downstream air, which is known to be a huge health risk in hospitals (Price, Simmons, Crow, & Ahearn, 2005).

Polyurethane is a synthetic polymer which forms from the reaction of a polyl (a long chained alcohol) with a diisocyanate. Depending on the type of polyl used, different types of polyurethane can be formed. The reticulated type of polyurethane foam is a porous, low-density type of foam characterised by a three-dimensional skeletal structure lacking membranes between the strands (Polyurethane Foam Association, 1994). The porosity of the foam is typically 95% but it can be as high as 98% (Gliganic, 2008). Currently reticulated foam is used in water filters and air filters found in air-conditioning units. No previous studies have adequately assessed the true potential of reticulated polyurethane foam as an air filter against fungal spores.

In light of the above, the aim of this study was to assess the air filtration capabilities in terms of fungal spores of a selection of polyurethane foams of differing combinations of pores per inch (PPI) and thickness so as to identify the foam PPI and thickness combination that is most effective for filtering fungal spores in air environments of different temperature and relative humidity.

2. Materials and methods

2.1. Sample collection

Outside air at the area of Msida in Malta (site of the Faculty of Health Sciences, University of Malta) was selected as the source of fungal spores. A filtration manifold with 3 ports was attached to an air flow meter which was in turn attached to a vacuum pump (Sartorius AG, Goettingen, Germany). The vacuum pump was subsequently connected to a power supply. The components were linked together by means of rubber tubing (See Fig. 1). Sterile cellulose nitrate membrane filters of pore size 0.45 μm were placed on the base of each manifold port (Sartorius AG, Goettingen, Germany). The ends of the tubing and the entirety of the setup were sealed by means of multiple sheets of parafilm M sealing film (Bemis NA, Neenah, Wisconsin) so as to ensure that the setup was thoroughly air-tight. A lack of air-leaks was confirmed by closing each of the valves of the 3 manifold ports, switching on the vacuum pump and noting an absence of air flow in the flow meter.

Three acrylonitrile butadiene styrene (ABS) plastic adapters, generated by a 3D printer, were designed in a manner that allowed for them to be inserted into the manifold ports while providing a platform into which any foams under assessment could be placed. The dimensions of the platforms were 45 mm x 45 mm x 20 mm. The adapters were sealed to the 3 manifold ports by means of further sheets of parafilm M sealing film.

Membrane filters were put into place by means of sterilised forceps and the adapters were duly sealed to the body of the manifold using sheets of parafilm M. The sampling of air occurred as the vacuum pump was switched on, air was drawn through foams supported by the adapters in the manifold ports, and spores were collected on the membrane filters. The necessary volume of air was sampled by running the vacuum pump for a period of time while the flow rate was measured by an air flow meter. Upon completing the sampling, the membrane filters were placed onto a separate Dichloran Rose Bengal Agar (DRBA) (Sigma Aldrich, St. Louis, USA) with Chloramphenicol plate. The inoculated plates were incubated in a cooling incubator (LeeC P3C, Leec Limited, UK) at 25 °C for 72 h. Following the incubation period, the number of colony forming units present on the membrane filters was counted.

A pilot study was performed to determine the ideal volume of air (or rather the minimum number of spores) to be sampled for the evaluation of the filtration aptitude of the foam materials. The volume of air sampled had to be large enough to allow some fungal spores to pass through the foam samples in order to reveal the different filtering capabilities of the different foams. The sampled volume of air had to also yield a countable number of colonies upon culturing. Therefore, the volumes of air sampled varied between 10 L and 1000 L. All experiments were performed on 3 separate days by running triplicates each time.

2.2. Evaluation of air filtration capabilities of polyurethane foams

The polyurethane foam samples (Articoli Resine Espanse [ARE], Italy) were cut using a Proxxon Thermocut 230/E (Proxxon, Föhren, Germany). Sheets of foam were cut along the direction of maximum cell elongation to thicknesses of 15 mm and 20 mm. Four polyurethane foams were selected for evaluation of air filtration capabilities against fungal contamination: 50 PPI foam of 15 mm thickness, 50 PPI foam of 20 mm thickness, 90 PPI foam of 15 mm thickness and 90 PPI foam of 20 mm thickness.

The experimental runs carried out consisted of the use of all 3
manifold ports for both the analysis of the foams’ filtering capabilities and controls. These runs differed in that foam analysis entailed positioning foam samples in the adapters when sampling air whereas the controls involved sampling air in the absence of these foam samples. For the control, the volume of air filtered was subdivided between the 3 manifold ports such that CFU’s of the control would be obtained by adding the CFU’s grown on the membrane filters of each individual manifold port.

When the foam samples were being analysed, the area around their periphery was made air-tight by the application of a generous amount of petroleum jelly to ensure that all the air being sampled was passing through the foam. Furthermore, in this case, 3 times the amount of air used in the control was sampled. In this way, the volume of air used in the control passed from each manifold port (assuming that equal amounts of air pass through each port).

The assessment of each foam consisted of three tests: a control run, a run to sample air through 3 identical foam samples (1 on each manifold port), and another control run, in that order. The captured spores were then calculated based on the differences between the control and the foam samples. This was repeated on three separate days for each of the polyurethane foams. Fresh foam samples were used each time (the foam samples were not re-used).

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**Fig. 1.** Components of air filtration experimental setup.

**Fig. 2.** Scatter Plot illustrating the variability of CFU counts at different sampling volumes.
Crucially, the temperature and relative humidity at the time of sampling was monitored and noted during all the runs using a Pro'sKit digital temperature and humidity meter.

2.3. Identification of fungal organisms trapped by the foam filters

Once the quantification of the colony forming units was carried out, the CFUs present were described accordingly. Each physically different CFU was sub-cultured onto fresh Sabouraud Dextrose Agar with Chloramphenicol (SDC) plates by stab inoculation to obtain pure fungal cultures. Sub-culturing was performed by collecting a visible amount of fungal material from the CFU on the DRBC plate with a heat-sterilised straight wire and stabbing (inoculating) an SDC plate at 4 ends. The plates were subsequently incubated at room temperature for 5–7 days.

The sub-cultured colonies were observed following the incubation period. If a plate was found to be contaminated, the desired CFU was sub-cultured onto a new SDC plates and re-incubated as required. Pure fungal cultures were initially assessed macroscopically by describing the colony present in terms of colonial morphology, colonial colour and diffusible pigment.

The pure fungal colonies were identified by means of microscopic examination of their conidia and conidiogenesis. The colonies were assessed microscopically by the adhesive tape mount technique using Lactophenol Cotton Blue (LPCB) dye as a mounting fluid. Occasionally, a lack of distinctive conidia or asexual structures was present on the adhesive tape mount. This equated to the organism remaining unidentified such that a means of promoting the production of conidia was required. This involved sub-culturing the unidentified organism onto Potato Dextrose Agar (PDA) and/or Corn Meal Agar (CMA) by stab inoculation. The media were incubated at room temperature for 24–48 h and once sufficient growth was present, the organisms were examined microscopically by the adhesive tape mount technique discussed above. Organisms which were still not producing conidia were incubated further and the adhesive tape mount technique was repeated after a few more days. Fungi which were persistently unable to produce conidia were naturally described as such.

2.4. Scanning electron microscope (SEM) imaging

Micrographs of the 50 PPI and 90 PPI polyurethane foams, at a magnification of 100× were captured using a Carl Zeiss Merlin Field Emission scanning electron microscope (SEM) with an InLens signal detector, a scan speed of 8 (the rate by which the image is scanned; in this case the SEM takes about 10 μs per line) and an EHT (Extra High Tension) of 3.00 kV. The foam samples were cut in cubes having a size of approximately 5 × 5 × 5 mm, making sure that the surface being examined was that of the foam’s rising direction. The sides and bottom of the cubes were covered with carbon tape.

The thicknesses of 20 randomly selected ribs of both of the foams were measured from the micrographs using an SPM data visualization and analysis tool, Gwyddion 2.4. The measurements obtained were converted to μm through:

\[ m_y = \left( \frac{p_m}{s_m} \right) s_v \]
where \( m_r \) is the measurement in micrometers, \( p_m \) is the measurement of the rib thickness from the micrograph, \( s_m \) is measurement of the scale and \( s_v \) is the scale value.

### 2.5. Data analysis

In order to investigate the effects of the thickness and the conventional foam PPI of the foam samples on the air filtration efficiency, the two independent variables were scaled and weighted according to the following formula:

\[
\text{Scaling and Weighting of Foam PPI or Thickness Measurements} = \left( \frac{\text{Original Measurement} - \text{Minimum Value}}{\text{Maximum Value} - \text{Minimum Value}} \right) \times \text{Weight Factor}
\]

The weight factors were selected in such a way as to obtain the highest correlation value (\( r^2 \) value) for the sum of the two modified independent variables against the air filtration efficiency. The effect of the two variables on the efficiency was studied by combining the conventional foam PPI and thickness into a single variable and attempting to correlate this variable with the air filtration efficiency. The variation of the number of CFUs captured with the ambient temperature and percentage humidity was also investigated in order to determine whether these environmental variables affect both the number of spores in the air (which is expected as per the pilot study), as well as their likelihood of being trapped by the polyurethane foams.

### 3. Results

#### 3.1. Selection of the volume of air to be sampled

The pilot study was performed to determine the volume of air to be sampled when carrying out the experiments. In other words, the minimum number of spores that needed to be collected in the control so as to obtain coherent results that were independent of the CFU count number needed to be evaluated. Initial sampling of different volumes of air on three separate days gave a clear indication that variability of CFUs/L was very large when small volumes of air were sampled (or rather when small number of CFUs was collected). This variability seemed to decrease at larger sampling volumes (larger number of CFUs collected). Fig. 2 demonstrates the variability of CFUs/L obtained at different volumes of sampling. Each data point represents a CFU count obtained after sampling a specific volume of air. The three readings obtained when sampling 10 L, 20 L, 40 L, 60 L or 80 L of air are considerably dispersed across the range of CFU/L at any given volume. Alternatively, the CFU/L results generated when sampling 100 L, 200 L, 300 L, 400 L or 1000 L of air are not highly variable at any given volume in terms of CFUs/L.

As is evident in Fig. 2, it was opted to sample on the side of caution, i.e., minimum variability, for a volume of at least 400 L. By using this volume an adequate number of CFUs would be of 10–60 CFUs on any given membrane filter. Note that as indicated in the Methodology section, the control is obtained by addition of three separate membrane filters found in each of the three manifolds ports. The pilot study was performed between August and
September in the region of Msida, Malta. Preliminary results have shown that seasonality and location enhance the microbial variability.

3.2. Assessing the efficacy of foam filters

Fig. 3 illustrates the amount of fungal spores captured on the membrane filters with each of the tested foams. The 50 PPI foam of 15 mm thickness significantly reduced the amount of fungal spores in the sampled air on day 2, but this was not evident on days 1 and 3. As opposed to the 50 PPI foam of 15 mm thickness, the 50 PPI foam of 20 mm thickness significantly reduced the amount of fungal spores in the sampled air on days 1, 2 and 3. The 90 PPI foam of 15 mm as well as the 20 mm thickness foam significantly reduced the amount of fungal spores in the sampled air in all days.

The percentage of fungal spores eliminated from the air by the foam on each day was calculated using the following formula:

\[
\text{Percentage Efficiency} = 100 \left( \frac{\text{Average CFU/L of Foam Analysis}}{\text{Average CFU/L of Control}} - 1 \right)
\]

Note by Percentage efficiency one understands the % of CFUs eliminated from the air.

The results obtained are shown in Fig. 4, which illustrates the filtration efficiency of each of the foam materials which were analysed.

The efficiency of the 90 PPI foam of 20 mm thickness is not significantly different to that of the 90 PPI foam of 15 mm thickness, but it is significantly different to that of the 50 PPI foams. When the efficiency of the 90 PPI foam of 15 mm thickness was compared with that of the 50 PPI foam of 20 mm thickness no significant differences were observed while it is significantly different to that of the 50 PPI foam of 15 mm thickness. It is evident that the efficiency of the 50 PPI foam of 15 mm thickness is significantly less than that of the other foams.

3.3. Assessing the effect of foam properties and environmental conditions on the foam efficacy

The relationship between the sum of the scaled and weighted foam thickness and conventional PPI, and the air filtration efficiency of the foam is shown in Fig. 5.

In order to obtain the maximum \( r^2 \) value, the weight factor of the conventional PPI was set to be twice as large as the weight value for the foam thickness. This value was obtained by varying the weight factor linearly until the highest \( r^2 \) value was obtained. The \( r^2 \) value of 0.8231 obtained indicates that both the conventional foam PPI and its thickness contribute positively and linearly (within the range tested) to the air filtration efficiency of the foam material, and that these two variables may be considered to account for a very high (~80%) percentage of the observed variance in CFUs, the rest being attributable to unknown causes such as natural variation in the number of spores in the air.

In order to check whether environmental factors affect the filtration capabilities of the foams, the temperature and relative humidity of the air sampled was measured during all experiments. These were then plotted against the measured filtration efficiency, in order to ascertain whether there is a trend (Fig. 6).

There seems to be next to no effect of the temperature or relative humidity on the filtration efficiency (see Fig. 6). In both figures a data point corresponding to a negative efficiency is present. These
Fig. 6. Relation between filter efficiency (%) versus temperature (°C) (left) and filter efficiency versus Relative Humidity (%) (right).
points have likely occurred due to the natural variability of the quantity of fungal spores in air in addition to the fact that it occurred with the least efficient foam.

3.4. Organism specific filtration efficiency of foam filters

Based on the microscopic analysis discussed in Section 2.3 the fungal organisms trapped by the tested reticulated polyurethane foam filters and the corresponding levels are presented hereunder:

- *Cladosporium* spp. (1.26 × 10^{-2} to 3.82 × 10^{-2} CFUs/L)
- *Penicillium* spp. (0–2.10 × 10^{-2} CFUs/L)
- Yeasts (1.88 × 10^{-3}–4.86 × 10^{-3} CFUs/L)
- *Geotrichum candidum* (0–1.25 × 10^{-3} CFUs/L)
- *Aspergillus niger* (0–2.50 × 10^{-3} CFUs/L)
- *Aspergillus terreus* (0–3.75 × 10^{-3} CFUs/L)
- *Aspergillus flavus* (1.00 × 10^{-3}–1.00 × 10^{-2} CFUs/L)
- *Aspergillus fumigatus* (0–1.25 × 10^{-3} CFUs/L)
- *Alternaria alternata* (0–1.81 × 10^{-3} CFUs/L)
- *Fonsecaea pedrosoi* (1.25 × 10^{-3}–2.50 × 10^{-3} CFUs/L)
- *Rhizopus oryzae* (0–1.25 × 10^{-3} CFUs/L)
- *Zygomycete* spp. (0–1.25 × 10^{-3} CFUs/L)
- *Sepeodon* spp. (0–1.25 × 10^{-3} CFUs/L)

In addition, a considerably large number of colonies remained unidentified due to an absence of conidia upon microscopic examination (range of 2.50 × 10^{-3} to 2.00 × 10^{-2} CFUs/L). Other colonies which appeared as ‘glossy’ circles on the cultured membrane filters remained unidentified as they were unable to grow a sufficient amount of fungal material in order for a macroscopic description to be made and for sub-culturing to be carried out (range of 2.58 × 10^{-2} to 6.29 × 10^{-2} CFUs/L).

3.5. Scanning electron microscope (SEM) micrographs

Micrographs of the 50 PPI and 90 PPI conventional polyurethane foams, at a magnification of 100×, were captured. The SEM micrographs of each of the foams are displayed in Figs. 7 and 8.

The thickness of 20 randomly selected ribs of both of the foams were measured. The results obtained were recorded in the table below (See Table 1).

The average rib thickness of the 50 PPI foam was found to be 53.65 μm with a standard deviation of 6.67 whilst that of the 90 PPI foam was established to be 37.15 μm with a standard deviation of 6.75.

4. Discussion

Most of the foam materials were found to be significantly effective at reducing the number of fungal spores in the filtered air. A clear trend is visible in Fig. 4, whereby the filtration efficiency of the conventional polyurethane foams increases with increasing PPI and foam thickness. An increase in PPI results in (1) smaller pores sizes, (2) a higher amount of pores and (3) thinner ribs, all of which aid the filtration process. Thinner ribs have such an effect as they increase the inertia on the spores passing through them by way of the larger surface area to volume ratio they provide. Studies show that pore size usually affects the initial and final filtration efficiencies, while the rib diameter has a significant effect on the rate of increase of filtration efficiency (Seok, Chun, Song, & Lee, 2015).

When correlating the conventional foam PPI and thickness with the efficiency of the foam material, the PPI was weighted to be 2 times more relevant than the thickness in determining the filtration efficiency of the foam material. This is likely to be the case since an increase in PPI results in more ribs and cells in a given area, and also thinner ribs.

Utilising a polyurethane foam filter as an air filter in food processing/storage facilities or hospitals would require that it be of a relatively high PPI. Although important, the thickness is not necessarily the priority in the design of the filter. A well-designed conventional polyurethane foam filter would provide a useful filtering tool, as presently demonstrated by its use in car filters and HVAC systems (New Dimension Industries, 2015).

Studies show a major disagreement as to the nature of the relationship between the seasonal variation of fungal spores and the environmental variables. Some studies found that the number of airborne spores was positively correlated with relative humidity but negatively correlated with temperature such that higher numbers were observed during autumn and winter than in spring and summer (Asan, Sarica Okten, & Sen, 2010; Bartzokas, 1975). Alternatively, other studies observed that a larger quantity of airborne fungal spores was present during the warmer and more humid summer months than during the winter season (Ceter & Pinar, 2006; Oliveria, Ribeiro, & Abreu, 2005; Shelton, Kirkland, Flanders, & Morris, 2002).

The two variables that were measured were the temperature and relative humidity. Neither of the two variables seemed to have...
an effect on the filtration efficiency of the foam materials. Unsurprisingly, both of these variables were found to influence the CFU count in the control, in a manner whereby the number of fungal spores in the air increased with an increase in the temperature and/or relative humidity. This partially explains why a greater diversity of organisms was observed in the summer season, than in the colder and less humid winter months.

5. Conclusions

In this work the air filtration capabilities in terms of fungal spores of a selection of polyurethane foams of differing combinations of pores per inch (PPI) and thickness were assessed. It was found that apart from the 50 PPI foam of 15 mm thickness, all the foam samples were effective at significantly reducing the number of spores. The PPI was found to be 2 times more influential on the efficiency of the foam material than the foam thickness. The higher number of pores present and the decrease in thickness of the ribs composing the microstructure of the foam explained the aforementioned observations.

Future studies could involve performing an in vitro study of the filtration capacities of the foams prior to analysing them in terms of outside air filtration. This would involve preparing a suspension containing a known quantity of genus/species-specific fungal spores and passing them through the foam materials being analysed in a closed system. This would provide a considerably more apt ground for comparison between the polyurethane foam under test and other established filtration materials.

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References


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