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INSTITUTO DE CIENCIAS BÁSICAS E INGENIERÍA

ÁREA ACADÉMICA DE QUÍMICA

PROPUESTA DE UN PROCESO BIOLÓGICO PARA EL TRATAMIENTO DE EFLUENTES CON BAJA RELACIÓN CARBONO:NITRÓGENO − CASO DE LA INDUSTRIA ACUÍCOLA

TESIS QUE PARA OBTENER EL GRADO DE:

MAESTRO EN QUÍMICA

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P R E S E N T E

Por este conducto le comunico que el jurado asignado al Candidato a Maestro en Química, **Q. A. Juan Ramírez Godínez**, quien presenta el trabajo **"Propuesta de un proceso biológico para el tratamiento de efluentes con baja relación Carbono:Nitrógeno-caso de la industria acuícola"**, después de revisar el trabajo en reunión de sinodales, ha decidido **autorizar la impresión** del mismo, una vez realizadas las correcciones que fueron acordadas.

A continuación se anotan las firmas de conformidad de los integrantes del jurado:

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Sin otro particular, reitero a Usted la seguridad de mi atenta consideración.

A T E N T A M E N T E
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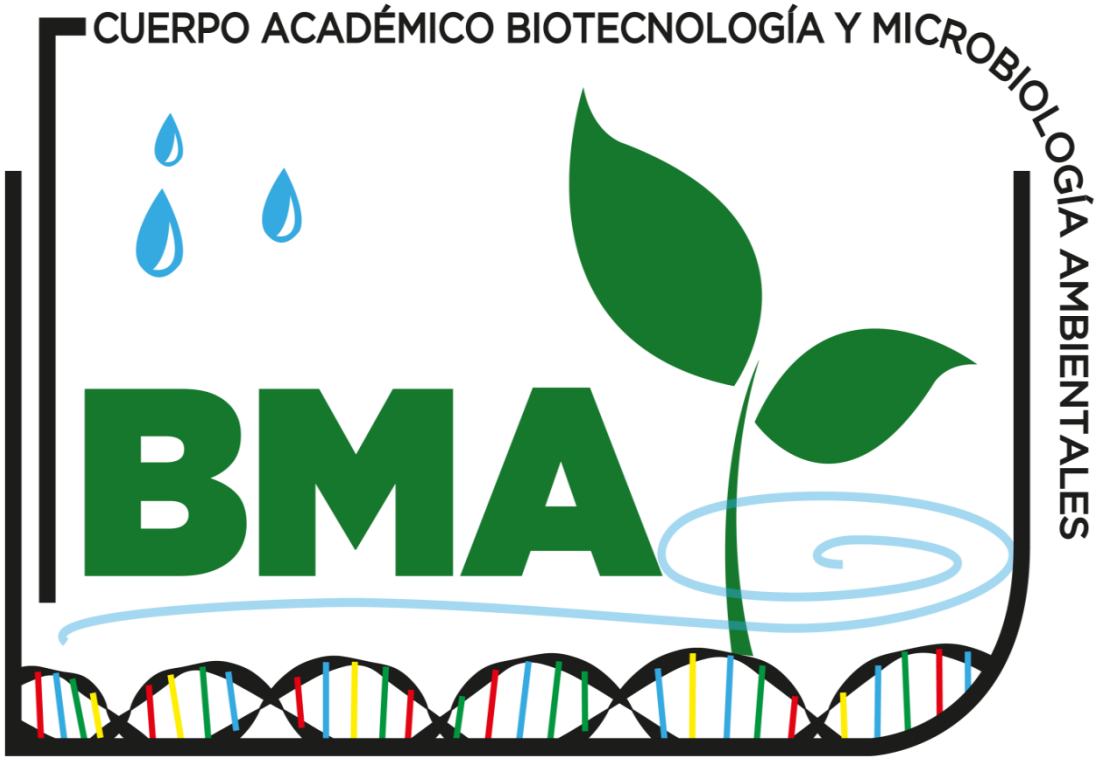

Este proyecto se realizó en los laboratorios 9 y 10 del Centro de Investigaciones Químicas.

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ÍNDICE

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Capítulo 1
Introducción

La acuacultura es la actividad mediante la que se crían peces y moluscos con la finalidad de obtener estos productos de una forma más rápida. A causa del descenso a nivel mundial de las poblaciones de peces que habitan en los océanos[1], el desarrollo de esta actividad ha crecido considerablemente. Así, los peces producidos por vía acuícola representan más de una cuarta parte de todo el pescado consumido por seres humanos, debido además a su bajo costo y a que representan una fuente importante de proteína, lípidos y de una amplia variedad de nutrientes.

Uno de los mayores problemas ambientales ocasionados por esta industria es la descarga de efluentes sin tratar, que contienen restos de alimento no ingerido, heces fecales, metabolitos y compuestos destinados a aumentar la producción. Este problema es grave porque estos efluentes tienen como destino final cuerpos de agua, en donde afectan negativamente a los ecosistemas.

Por lo anterior, en el capítulo 2 y 3 se presentan la justificación y los objetivos de este proyecto, respectivamente. Entre estos destaca el objetivo general, que fue proponer un proceso de tratamiento basado en biofiltros para efluentes acuícolas que tengan una baja relación carbono:nitrógeno que permita su recirculación en el sistema productivo.

El capítulo 4 es una revisión bibliográfica que presenta los contaminantes más comunes de los efluentes acuícolas; entre ellos se encuentran contaminantes convencionales (sólidos suspendidos totales y materia orgánica), nutrientes (nitrógeno y fósforo), así como pesticidas, metales pesados y contaminantes emergentes (antibióticos y hormonas). También se muestran los sistemas de recirculación, que integran el tratamiento y la reutilización del agua en el proceso. Estos sistemas constituyen una alternativa para la

[1] Naylor L.R., Goldburg R.J., Primavera J.H., Kautsky N., Beveridge M.C.M., Clay J., Folke C., Lubchenco J., Mooney H. y Troell M. (2000) Effect of aquaculture on world fish supplies. *Nature* 405, 1017-1024.

prevención de la contaminación del agua, ya que permiten disminuir el volumen de las descargas y evitar problemas ambientales como la eutrofización.

Con la finalidad de proponer un sistema de recirculación de agua residual acuícola, se caracterizaron fisicoquímica y microbiológicamente los efluentes de la Granja Integral de Policultivo, ubicada en Tezontepec de Aldama, Hidalgo. Los resultados se exponen en el capítulo 5. Se determinó que los efluentes superan los valores guía para el reúso del agua en términos de sólidos suspendidos totales, DBO_5 y Nitrógeno Total Kjeldahl (NTK). Se encontró también que el tratamiento biológico por medio de biofiltros que completan la secuencia nitrificación-desnitrificación es una forma económica y factible para cumplir con los valores guía de reúso. Para alcanzar este objetivo, sin embargo, es necesario adicionar una fuente externa de C, ya que la relación C:N es baja y por tanto desfavorable para el tratamiento biológico.

Los biofiltros son sistemas que, mediante el contacto de aguas residuales con biomasa adherida a un soporte fijo, reducen la carga orgánica de estas. Si se diseñan para tal fin, permiten la remoción biológica de nitrógeno de manera simultánea. Uno de los materiales de soporte más utilizados es el carbón activado granular (CAG), y por este motivo decidimos evaluar la capacidad de este material para remover los principales contaminantes de los efluentes acuícolas. El capítulo 6 presenta los resultados de esta evaluación. Primero, se determinó la capacidad de adsorción del CAG usando una molécula modelo, el azul de metileno. Para ello se realizaron pruebas estáticas por lote y los resultados se ajustaron al modelo de Langmuir. Segundo, se realizaron pruebas dinámicas para conocer las cinéticas de adsorción de la molécula modelo. Los resultados de las pruebas dinámicas se ajustaron a un modelo de pseudo segundo orden. Por último, para estudiar la capacidad de remoción de los contaminantes del efluente de la Granja Integral de Policultivo, se hicieron ensayos continuos en una columna empacada con CAG. En estos ensayos se midieron pH, SST, DQO, UV_{254}, NTK, $N\text{-}NH_4^+$, P-total y coliformes fecales.

Para el funcionamiento del biofiltro desnitrificante, será necesaria la adición de una fuente exógena de carbono. Por tal motivo, el capítulo 7 muestra la evaluación de tres materiales

naturales (aserrín, granos de cebada y cáscara de cacahuate). Para tal propósito se midió la cantidad de carbono orgánico, compuestos nitrogenados (NTK, $N\text{-}NH_4^+$, NO_2^- y NO_3^-) y fósforo total liberada en pruebas realizadas durante 50 días. Además se estudiaron las características de la materia orgánica lixiviada a partir de los tres materiales mediante espectroscopía IR y análisis elemental, y se seleccionó el material más adecuado como donador de carbono.

Finalmente, en el capítulo 8 se hace un recuento de las conclusiones derivadas de la experimentación, mientras que el capítulo 9 muestra las perspectivas que deberán considerarse para la implementación del sistema propuesto de tratamiento biológico de efluentes acuícolas.

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Capítulo 2
Justificación

Según la FAO[2], desde finales del siglo XX y hasta la fecha, la acuacultura ha constituido un mecanismo alterno de suma importancia para la producción de alimentos a nivel mundial. En México, esta actividad surgió como apoyo social para las comunidades rurales, con el objetivo de incrementar el consumo de proteína animal y mejorar la calidad de vida de las personas.

A pesar de ser un estado carente de litorales, en los últimos años Hidalgo se ha convertido en un productor acuícola destacado. Este crecimiento se debe en gran parte a trabajos comunitarios organizados por sectores sociales e impulsados económicamente por dependencias del orden gubernamental.

Sin embargo, uno de los mayores problemas ambientales ocasionados por esta industria es la descarga de efluentes sin tratar, que representan el 40% del volumen total descargado por las industrias en México[3]. Estos efluentes degradan la calidad del agua de los cuerpos receptores debido a que contienen cantidades considerables de nutrientes, en particular compuestos nitrogenados, que pueden conducir a su eutrofización. En un esquema de uso sustentable del agua, es necesario desarrollar e implementar sistemas de recirculación integrados al proceso productivo, razón por la cual en este proyecto se propone un sistema de tratamiento para el manejo de efluentes acuícolas que presenten una baja relación carbono:nitrógeno.

[2] FAO (2012) Acuacultura. Food and Agriculture Organization. Recopilación de datos disponible en: http://www.fao.org/fishery/aquaculture/es

[3] CNA (1998) Inventario Nacional de Descargas de Aguas Residuales. Comisión Nacional de Agua, México D.F.

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Capítulo 3
Objetivos

3.1 General

Proponer un proceso de tratamiento basado en biofiltros para efluentes acuícolas con una baja relación carbono:nitrógeno que permita su recirculación en el sistema productivo.

3.2 Específicos

- Examinar los requisitos de calidad establecidos por la industria acuícola para el agua de proceso que ha sido recirculada.

- Caracterizar fisicoquímica y microbiológicamente efluentes de la industria acuícola para proponer un tratamiento adecuado.

- Evaluar el uso de carbón activado granular (CAG) en el tratamiento de efluentes acuícolas.

- Seleccionar un material natural entre tres posibles fuentes (aserrín, granos de cebada y cáscara de cacahuate) que sirva como donador exógeno de carbono en un biofiltro desnitrificante.

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Capítulo 4

Recirculating Systems for Pollution Prevention in Aquaculture Facilities

Como todas las formas de producción de alimento, la acuacultura, definida como la cría de peces y moluscos, tiene numerosos impactos ambientales. La contaminación del agua es el más significativo, ya que los efluentes acuícolas contienen heces y alimento no ingerido que afectan a los cuerpos de agua receptores cuando se descargan sin tratamiento alguno. En dichos efluentes se reporta la presencia de contaminantes convencionales (sólidos suspendidos totales y materia orgánica) y nutrientes, así como pesticidas, metales pesados y contaminantes emergentes (antibióticos y hormonas). Con base en esta composición, se ha observado que los sistemas acuícolas de recirculación (*i.e.*, sistemas que integran el tratamiento y la reutilización del agua en el proceso) son una alternativa para la prevención de la contaminación del agua, ya que permiten disminuir el volumen de las descargas y evitar problemas ambientales como la eutrofización. A partir de la revisión bibliográfica realizada, se concluye que los biofiltros de carbón activado son una tecnología adecuada para alcanzar un nivel de calidad del agua que sea compatible con prácticas acuícolas sustentables.

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Recirculating systems for pollution prevention in aquaculture facilities

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Abstract

As all other forms of livestock production, fish farming has numerous environmental impacts. Water pollution is one of the most significant outcomes, since aquaculture effluents contain non-ingested food and fish dregs that affect the receiving water bodies when discharged without any treatment. Conventional pollutants (suspended solids, dissolved organic matter and nutrients), as well as pesticides, heavy metals and emerging pollutants (as antibiotics and hormones), are commonly found in these effluents. Recirculating aquaculture systems (RAS, systems that integrate the treatment and the reuse of water in the process) are an invaluable alternative for preventing water pollution by diminishing both the volume and the eutrophication potential of the effluents. Based on our review of the extant literature in the field, we conclude that activated carbon-based biofilters are a favorable technology to achieve a level of water quality that is compatible with environmentally-sound aquaculture practices.

Keywords: Fresh water production; Biofilter; Nitrogen removal; Biological activated carbon

1. Sustainability of aquaculture

Fish is an exceptional source of good-quality proteins, lipids and a wide variety of essential nutrients. Production of farmed fish, or aquaculture, is probably the fastest growing food sector worldwide, as now it accounts for nearly 40% of the world fish production [1]. Aquaculture production has expanded 12-fold in the last three decades (1980–2010) [1], and the reliance on farmed fish will certainly increase alongside with world population [2].

Until two decades ago, when extensive technologies prevailed, aquaculture was considered an environmentally-sound activity. Several traditional techniques even functioned as efficient water treatment systems, thereby contributing to the abatement of pollution [3]. But recently, with the adoption of more intensive production systems, the sustainability of aquaculture has been questioned. Environmental concerns arise from both the increased use of resources (as land, water, feed and energy) and the concomitant waterborne and airborne emissions of the farms. The risks inherent to aquaculture [4-5] can be summarized as in the following list.

- Habitat alteration or destruction
- Generation of organic-rich sediments
- Excessive freshwater consumption
- Modification of water temperature and flow rate profiles
- Water pollution
- Modification of the biotic index
- Transmission of infections from farmed organisms to wild stock
- Emergence and spread of antibiotic resistance
- Genetic risk of escaped culture animals
- Introduction of exotic species
- Diminution of wild fish stock for farming carnivorous species
- Multi-use conflicts for resources

However, for some authors, even larger-scope impacts of aquaculture should be taken into account, such as greenhouse gases originating from energy consumption and their contribution to global warming, ocean acidification and ozone layer depletion [6].

Here we examine the effects of aquaculture in the quality of receiving water bodies, with emphasis in the impacts of freshwater fish production in ponds. This review examines the water quality requirements of the industry

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and summarizes the extant literature concerning the pollutants expected to be present in the effluents from intensive aquaculture facilities. We present also the recycling aquaculture systems (RAS) as an efficient alternative for pollution prevention in these facilities.

2. Farmed fish production and water quality

An aquatic farm (Figure 1) has water quality levels to maintain, which are very dependent on the species cultivated. The main requirements concern dissolved oxygen, pH, ammonia and nitrites [3]. In salmonid culture, dissolved oxygen levels are not allowed to be less than 5 mg/L for more than a few hours. Although carp and tilapia in farms can tolerate lower concentrations (ranging from 3 to 4 mg/L), the optimum levels of dissolved oxygen are higher, and so the desirable range is usually above 5 mg/L. For pH values, the desirable range for fish production is 6.5 – 9.0 [3].

Toxicity of ammonia is generally attributed to the concentration of the unionized ammonia molecule (NH_3), due to its ability to move across cell membranes [7]. Median lethal concentrations (LC_{50}) over a 96-hour period of exposure to unionized ammonia have been established for rainbow trout (0.32 mg/L), bluegill (0.4 – 1.3 mg/L) and channel catfish (1.5 – 3.1 mg/L) [8]. Since chronic exposure to low concentrations of ammonia may reduce growth and also increase the susceptibility to diseases, some authors consider the maximum tolerable concentration to be 0.1 mg/L, although the preferred level is lower (the EPA standard for rainbow trout is 0.02 mg/L) [8]. The content of unionized ammonia is determined by the concentration of total ammonia nitrogen (TAN), pH and temperature. In this way, at a TAN of 5 mg/L and pH of 9.0, typical fish would be dead in hours, while with pH less than 6.0, ammonia would have negligible impacts at the same TAN concentration [7]. Concerning nitrites, the suggested maximum level for prolonged exposure in hard freshwater is 0.1 mg/L [3]. The main mechanism of nitrite toxicity relies on the transformation of hemoglobin to meta-hemoglobin, which lacks the capacity to bind oxygen irreversibly [9].

Figure 1. Farmed carp production.

3. Pollution caused by freshwater aquaculture effluents

Modern aquaculture depends upon the supply of nutrient inputs. However, for some species, a large fraction of the food ration can remain uneaten (e.g., European eels and tilapias spill around 1 – 10% and 10 – 30% of the ration, respectively [4]). Thus, on the one hand, the rates of supply and assimilation of nutrient inputs are decisive factors of the farm outputs, in particular for intensive operations in open aquaculture systems [10]; on the other hand, overfeeding should be avoided due to its large impact on water quality.

Aquacultural wastes include all materials used in the process which are not removed from the system during harvesting [11]. These wastes are mainly associated to uneaten feed or excreta, chemicals and therapeutants added to the ponds, and can be discharged either in the sediments or in the farm effluents. Sediments are usually collected intermittently or at the end of the production cycle and consist of inorganic and organic particulate material. By contrast, effluents are commonly discharged on a continuous basis over the production cycle and contain both dissolved and particulate pollutants (inorganic and organic) [10].

Although the characteristics of aquaculture effluents are highly variable following the cultivated species, the type of production facility and the feed quality and management, some general features can be drawn (Table 1). In a general way, the quality of aquaculture effluents is rather comparable to raw surface water than to domestic or secondary effluents, with low contents of total suspended solids (TSS), organic matter, and total and ammonium nitrogen. However, these low levels of pollution are not conducive to easy treatment, at least concerning solids. In fact, it has been reported that the efficiency of sedimentation increases with higher concentrations of solids [11].

Table 1. Comparison between aquaculture effluents and other types of water.

Parameter	Domestic effluent	Domestic secondary effluent	Aquaculture effluent	Raw surface water*
TSS [mg/L]	400 – 500	30	5 – 50	50 – 400
TKN [mg/L]	300 – 400	20	3 – 20	7
N-NH_4^+ mg/L]	40 – 75	5	0.5 – 4.0	0.05 – 0.50
BOD_5 [mg/L]	300	20	0.2 – 0.5	2 – 4

* That requires treatment. Sources: [3, 13]

3.1. Conventional pollutants

Conventional pollutants (TSS, BOD_5 and nutrients) are mainly derived from feed, excreta and fertilizers. The main purpose of the addition of fertilizers is the stimulation of both phytoplankton growth and fish production. Inorganic compounds of N and P are among the most usual fertilizers, but K, trace metals, and silicates may also be present [12]. Since fertilizers increase the concentrations of nutrients in pond water, they may cause eutrophication in receiving water bodies.

Even though the concentrations of conventional pollutants are usually low, pond cleaning can increase them considerably. In a study examining the quality of effluents from a hatchery, TSS, BOD_5 and total phosphorus increased during cleaning from 1 to 88 mg/L, 3 to 32 mg/L and from 0.22 to 4.00 mg P/L, respectively [14].

Due to their content of nutrients, aquaculture effluents are well-suited for biological treatments (e.g., wetlands, biofilters or algae-based systems) and agricultural reuse (as in hydroponics and crop production). In the first case, it must be noticed that aquaculture discharges have nitrogen levels disproportionately high regarding carbon contents [4]. As balanced microbial growth requires a C:N ratio of about 100:10, biological treatment of aquaculture effluents is likely to involve the addition of exogenous carbon substrates.

3.2. Pesticides, heavy metals and emerging pollutants

Intensive aquaculture often relies on chemical additives for health management, manipulation of reproduction or growth promotion, among other purposes. Some pesticides commonly used are rotenone, simazine, 2,4-D, diquat and diuron [3, 10], essentially for weed control. Organophosphate compounds (as malathion and dichlorvos), carbamates and pyrethroids are also employed as parasiticides. A concern arises from their non-selective action and their long-term effects on pond productivity [3]. However, the concentration of pesticides in aquaculture effluents is scarcely reported, and there is a lack of information about their effects on non-target organisms.

Heavy metals can also be found in pond effluents because they are common constituents of proteinates and vitamin/mineral premixes (e.g., Cu and Zn [15]). But mainly, they can be added to ponds as oxidizing agents for controlling phytoplankton and pathogenic organisms (e.g., $KMnO_4$ [12]) or as algicides (e.g., $CuSO_4$ [12]). Although these metals tend to precipitate as bottom sediments, the applied doses should be surveyed to avoid any toxic effect in fish. For instance, $CuSO_4$ is frequently used for eradicating submerged weeds, but the safe Cu levels have not been fully established for chronic exposure [15]. In fact, sublethal effects of Cu such as reduced swimming speed, reduced feeding and growth inhibition have been widely reported in salmonids [15].

Nowadays, one of the main environmental concerns about aquaculture is the release of bactericides (glutaraldehyde, formalin), therapeutants (as malachite green and dipterex) and antibiotics (mainly tetracyclines, quinolones and β-lactams) to the aquatic media. Some of these compounds are added in appreciable amounts; for instance, glutaraldehyde and formalin are regularly added at concentrations of 1 – 10 mg/L to avoid the proliferation of pathogens [12]. In a survey of fish farms in England, contents as high as 15.20 and 0.61 mg/L of formalin and malachite green, respectively, were found [3]. It is worth to note that malachite green is environmentally persistent, mutagenic in rats and mice, cytotoxic to mammalian cells and carcinogenic to experimental animals [16]. Even though malachite green has been banned in several countries, it is still used in others due to its efficiency and low cost [16]. Antibiotics are found in the water of intensive farms rather than in extensive ones [17], most likely because in an intensive hatchery fish are subject to more stressors that decrease the ability of their immune system to deal with infections [18]. The concentrations measured for antibiotics are usually low (e.g., from 0.17 to 10 μg/L for oxytetracycline [19]), although they rise noticeably through prophylactic treatments. Ormetoprim content has been measured at 0.69 μg/L, but it can be found at levels as high as 12 μg/L during fish treatment [20]. The main consequence of the reliance of aquaculture on antibiotics is probably the augmented antibiotic resistance in fish pathogens, which raises the possibility for passage of their antibiotic resistance determinants to bacteria of land animals and human beings via the food chain.

Intensive fish farming is also a source of steroid hormones such as estrone, testosterone and androstenedione [21]. In fact, estrone has been pointed out as the most important natural endocrine disrupting compound found in natural water due to its ubiquity and estrogenic potency (higher than that of nonylphenol) [22]. Steroids are present in the blood plasma of fish and can be excreted via urine or bile, mainly during periods of reproduction [21]. The contents detected (of about 1 ng/L) of these emerging pollutants in aquaculture effluents are similar to those found in domestic secondary effluents and high enough to lead to adverse reproductive effects in aquatic species as trouts [21-22]. However, the removal and the effects of hormones in the usual treatment systems of aquaculture effluents have not been studied thoroughly yet.

4. Recirculating aquaculture systems (RAS)

The reduction of the wastewater volume is essential for enhancing the sustainability of fish farming, and recirculating aquaculture systems (RAS) have been proposed with this purpose. In these systems, water is partially reused in the process after undergoing a proper treatment, thereby reducing water usage and improving effluent quality. By means of the life cycle analysis methodology, RAS have been compared against a conventional flow-through system [23]; it has been found that RAS reduce water dependence by 93% in comparison to conventional systems. Moreover, RAS eutrophication potential resulted to be 26 – 38% lower than that of traditional systems.

RAS technology relies considerably on biological filtration as the mechanism for removing critical pollutants [24]. In a study following the oxytetracycline content in water of a sand biofilter-based RAS, peak concentrations of 0.39 – 0.72 ng/L were detected in the water both entering and leaving the biofilter only during the 10-day treatment of fish [25]. All through the therapeutic period, the amount of oxytetracycline discharged by RAS was considerably lower than that discharged by a conventional flow-through system [25].

In addition, through nitrification, biofilters are able to make recycled water suitable for fish production by oxidizing TAN to nitrates. In first generation-RAS, the maximum allowed concentration of nitrates steers the external water exchange rate [23]. But recent technological developments include a denitrification reactor for full nitrogen removal. As a result, last generation-RAS reduce water consumption, as well as the concentrations of nitrates and BOD_5 in the final discharge [23]. Although RAS are intended to reduce the water volume used, a minimum water exchange ratio must be maintained. By lowering the make-up water volume, an accumulation of growth inhibiting factors (e.g. fish-produced cortisol, bacterial metabolites and metals) is likely to occur. In a low water exchange RAS, the accumulation of phosphate, As and Cu led to higher mortality and reduced larvae length and body weight in the culture of carp [26].

Activated carbon-based biofilters are well-suited for RAS, because they offer the possibility of removing pollutants either by adsorption or by biological mechanisms such as biodegradation, nitrification or denitrification. It has been demonstrated that biological activated carbon filters (i.e., fixed beds of granular activated carbon supporting bacterial growth) can effectively remove (>90%) emerging pollutants such as pesticides, steroids, antibiotics and other persistent chemicals from water [27] and wastewater [28]. In this way, the accumulation of growth inhibiting factors in RAS could be avoided.

5. Conclusions

Aquaculture effluents contain low concentrations of conventional pollutants (TSS, organic matter and nutrients), pesticides, heavy metals and emerging pollutants. Biological treatments are environmentally-friendly alternatives for removing these pollutants in RAS and hence for preventing the pollution originated by this industry. To this end, activated carbon-based biofilters seem appropriate for minimizing water exchange ratios in RAS without compromising the quality of fish production by the accumulation of growth inhibitors. However, the typical unbalance between the contents of organic matter and nitrogen could require the addition of easily assimilable carbonaceous sources for achieving full nitrogen removal.

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Capítulo 5

Characterization of Aquaculture Effluents for In Situ *Treatment and Reuse*

Los efluentes que provienen de la acuacultura son una fuente importante de materia orgánica, sólidos suspendidos totales y nutrientes, que ocasionan problemas ambientales como la eutrofización si se descargan en el medio acuático sin tratamiento previo. Con la finalidad de proponer un sistema de recirculación de agua residual acuícola, se caracterizaron fisicoquímica y microbiológicamente los efluentes de la Granja Integral de Policultivo ubicada en Tezontepec de Aldama, Hidalgo. Los efluentes sobrepasan los valores guía para el reúso con respecto a sólidos suspendidos totales, DBO_5 y NTK. Sin embargo, la composición del efluente indicó que el tratamiento biológico es una forma económica y factible para satisfacer las directrices determinadas por la industria acuícola. Además, se observó que para lograr la eliminación completa de nitrógeno es necesario el uso de una fuente exógena de carbono biodegradable.

CHARACTERIZATION OF AQUACULTURE EFFLUENTS FOR *IN SITU* TREATMENT AND REUSE

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Abstract

Freshwater aquaculture effluents are an important source of organic matter, suspended solids and nutrients if they are discharged in the aquatic environment without prior treatment. In order to propose a recirculating aquaculture system (RAS, a system that integrates the treatment and the recycling of water in the process), the effluents from a polyculture farming system were characterized physicochemically and microbiologically. The effluents exceeded the reuse guideline values suggested for total suspended solids, BOD_5 and total Kjeldhal nitrogen. However, the composition of the effluent indicated that the biological treatment is an economical and feasible way to meet the guidelines for RAS. Also, it was observed that an external input of biodegradable carbon is necessary for the full removal of nitrogen (*i.e.*, involving nitrification and denitrification).

Introduction

The decrease of fish population all over the world is nowadays reflected in the importance of aquacultured species as an alternative source of good quality protein and lipids. In fact, the expansion of this sector of animal food production is the fastest worldwide [1]. The development of this industry has led to several environmental impacts, including conflicts with other economic activities due to the concurrence for available resources, a higher risk of diseases spread and genetic pollution [2]. However, the main effect occurs in the aquatic environment. Aquacultural effluents contain non-ingested food and fish dregs, and they affect the receiving water bodies when are discharged without any treatment. Suspended solids, dissolved organic matter, inorganic compounds (such as ammonium, orthophosphates, nitrites and nitrates), as well as pathogenic microorganisms, are commonly found in these effluents [2]. Particularly, the discharge of nutrients exceeding the assimilation capacity of the receiving aquatic bodies results in their environmental deterioration, in algal bloom episodes and eventually in their eutrophication.

The treatment and reuse of the effluents are essential for enhancing the sustainability of aquaculture. Recirculating aquaculture systems (RAS) are characterized by at least a biological reactor (usually a biofilter or a wetland) allowing organic carbon and nutrients to be removed before water returns into the production unit [3]. As a preliminary step towards the RAS design, the objective of this work was to evaluate the main physicochemical and microbiological characteristics of effluents arising from intensive polyculture farming.

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Materials and methods

Water sampling. The samples were obtained in different days (June 26 and October 8, 2012, and March 11, 2013) from the ponds of a polyculture farming system (*Granja Integral de Policultivo*, Tezontepec de Aldama, Hidalgo, Mexico; see Figure 1). Four to five individual samples were taken from different points of a same pond (for production mainly of *Cyprinus* spp.), and then combined to form a composite sample. One sample of the water entering to the pond was taken too (October 8, 2012). *In situ* measured parameters were pH, dissolved oxygen and temperature, which were assessed by using an Orion® multiparameter probe. For microbiological measurements, samples were stored in sterile flasks. The water samples were transported to the laboratory and preserved at 4°C until analysis. Microbiological analyses were performed in the same day of sampling.

Figure .1- Pond of the sampling site (Tezontepec de Aldama, Hidalgo, Mexico).

Physicochemical characterization of the pond water. Excepting where otherwise indicated, the parameters were measured according to the Standard Methods [4]. Alkalinity was measured by method 2320 and expressed as mg $CaCO_3$/L. Total suspended solids (TSS) were measured gravimetrically at 105 ± 5 °C (method 2540-D). Chemical oxygen demand (COD) was assessed after digestion of the samples by measuring spectrophotometrically the decrease in the Cr(VI) concentration at 600 nm (method 5520). Biochemical oxygen demand (BOD_5) was determined in the OxiTop® measurement system (WTW, Germany). Total organic carbon (TOC) was measured in a Shimadzu® TOC-V analyzer. For measuring total Kjeldhal nitrogen (TKN, method 4500-B), the samples were digested at 400°C and distilled in a Gerhardt® Vapodest 20 unit to transform organic nitrogen into ammonium ions, which were further measured by titration with 0.01N HCl. The same method was used for assessing N-NH_4^+ but omitting the digestion step. Unionized ammonia was estimated from the following equation by using the ammonium nitrogen content, the ammonium acidity constant (9.4 at 20°C) and the pH value of the samples [5].

$$\mathrm{N-NH_3} = \frac{\mathrm{N-NH_3} + \mathrm{N-NH_4^+}}{1 + 10^{(\mathrm{pKa-pH})}}$$

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The diazotization method was used for measuring $N\text{-}NO_2^-$ (4500-NO_2^-). A screening method (4500-NO_3^-), based on absorbance measurements at 220 nm, was used to estimate $N\text{-}NO_3^-$. Finally, the ascorbic reduction method (4500-P F) was employed to assess PO_4^{3-}.

Microbiological characterization of the pond water. Total cultivable bacteria were estimated by heterotrophic plate counts (HPC; NOM-092-SSA1-1994). Total and fecal coliforms were measured by membrane filtration-based techniques (APHA methods 9222 B and D, respectively). *Salmonella* spp., *Staphylococcus aureus* and *Vibrio cholerae* were detected in presumptive tests by using Mexican standard procedures (NOM-114-SSA1-1994, NOM-115-SSA1-1994 and NOM-016-SSA2-1994, respectively).

Results and discussion

Table 1 presents the characterization results for water both entering and leaving the culture ponds. Some values reported in the literature for aquaculture effluents and guideline values of water quality suggested for reuse systems [6] are also presented.

Table 1.- Physicochemical characterization of the pond water.

Parameter	Units	Influent	Effluent[A]	Literature values and references	Guidelines for RAS [6]
pH		7.5	7.8	7.3 [7] 7.7 - 8.6 [8]	6.0 - 9.0
DO	mg/L	ND	8	4.0 - 12.3 [8]	> 0.6
T	°C	22.1	22.4	12 - 30 [8]	-
Alkalinity	mg $CaCO_3$/L	45	63	151 - 302 [8]	> 20
TSS	mg/L	ND	614	53 - 2674 [8] 60 - 120 [6] 1000 [7]	< 10
COD	mg/L	59.6	208.2	9.3 [9] 200 - 800 [10] 240 [7]	-
BOD_5	mg/L	11	47.5	1.6 [6] 4 - 8 [11]	< 5
TOC	mg/L	ND	80.5	1.8 [12]	-
TKN	mg/L	ND	400.5	1.5 - 2.8 [11] 60 [7]	40
$N\text{-}NH_4^+$	mg/L	2.6	4.8	0.14 - 3.56 [8] 0.21 [9] 7 [7]	-
$N\text{-}NH_3$	mg/L	0.03	0.12	1.3 [6]	0.02 - 0.41
$N\text{-}NO_2^-$	mg/L	0.001	0.02	0.01 - 0.15 [8] 0.6 [6] 5 [7]	0.06 - 1.52
$N\text{-}NO_3^-$	mg/L	3.2	5.1	0.01 - 0.15 [8] 0.35 [9] 6.5 [6] 110 [7]	< 226
PO_4^{3-}	mg/L	1.4	11.9	0.4 - 6.8 [8] 50.4 [7]	-

[A]The results are the mean values calculated from two independent samples.
ND: Not determined value.

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The guidelines for water reuse consider that pH, DO, alkalinity, TSS, BOD_5, $N\text{-}NH_3$ and $N\text{-}NO_2^-$ are the limiting factors for maintaining a proper culture environment. This is especially important in the case of $N\text{-}NH_3$, because this species has a direct effect on the growth of aquatic animals as catfish [5]. Moreover, high unionized ammonia concentrations have an effect on the incidence of fish diseases, particularly at low DO levels [5]. Concerning $N\text{-}NO_2^-$, a concentration higher than 0.5 mg/L can be toxic for fish, mainly due to its role in the conversion of respiratory pigments (hemoglobin, hemocyanin) in forms unable to transport free oxygen (*i.e.*, meta-hemoglobin and meta-hemocyanin), which can cause asphyxia and ultimately death [13]. It has been also recognized that concentrations higher than 0.09 mg $N\text{-}NO_2^-$/L can cause toxic effects to salmonids and cyprinids. Since nitrate is relatively harmless to cultured aquatic organisms [10], the guideline value is rather high. However, if nitrates are allowed to accumulate in an aquaculture pond, toxic levels of nitrates could be produced, because nitrate-reducing bacteria are likely to proliferate in microsites lacking of oxygen (*e.g.*, at the bottom of the pond) [10]. A maximum level of 2 mg $N\text{-}NO_3^-$/L has been also proposed to protect the most sensitive species during long exposure [13].

It is worth noting that the effluent meets the reuse guideline values established for pH, DO, alkalinity, $N\text{-}NH_3$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$. Besides, the values of most of these parameters are similar to the literature data. However, the levels of TSS, BOD_5 and TKN exceed the recommended values for RAS, and they are higher than most of the published data. TSS could arise from resuspended sediments, fecal matter produced by fish, non-ingested feed or algal growth [6]. For these reasons, high TSS levels are positively correlated to pond-fish densities. Actually, the concentrations of nutrients increase along with harvest density too [11]. High values of both BOD_5 and TKN have been also associated with phytoplankton biomass and high contents of chlorophyll a; inorganic wastes produced by fish stimulate the development of phytoplankton that may produce two- to four-fold as much organic carbon as that coming directly from fish [8]. In this way, BOD_5 and TKN commonly rise as season progresses [11]. Since phosphates have not been reported as responsible for any adverse effect in aquatic organisms [8], they are not considered in the guidelines for water reuse. Nonetheless, an efficient RAS configuration should avoid the accumulation of phosphates to guarantee the long-term operation of the system.

The results of the microbiological evaluation of the pond water are shown in Table 2. Although it has been found that the density of bacteria in water determines their presence in the cultured fish [11], the reuse guidelines do not take into account microbiological parameters [6]. So, only published or suggested values are presented in Table 2 for comparison purposes.

The heterotrophic plate counts (HPC) and total coliforms densities in the effluents are consistent with other published values. Regarding to the content of fecal coliforms, the values measured for both the inlet and the effluent are considerably higher than the bibliography data. In aquaculture wastewater, fecal coliform counts have been related to harvest densities [12] and to the growing season [11]. Even though fecal coliforms are rather originated from warm-blooded animals (*e.g.*, livestock, pets or birds) and are not indicative of pathogenic waterborne bacteria [8], some authors suggest caution because

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there exists a risk of penetration into the fish muscles when the concentration of fecal coliforms exceeds $5x10^4$ CFU/mL [11]. For *Salmonella* spp., this risk has been estimated to occur at $1x10^3$ CFU/mL, and the potential of muscle invasion increases with the length of exposure to the contaminated water [14]. Therefore, Mara and Cairncross [14] advocate a limit value of 100 CFU/mL for aquaculture water.

Table 2.- Microbiological characterization of the pond water.

Parameter	Units	Influent	Effluent[A]	Literature values and references
HPC	CFU/mL	$3x10^4$	$6x10^4$	$1.7x10^3$ [12] $4x10^5$ [9]
Total coliforms	CFU/mL	$2x10^2$	$1.6x10^3$ [B]	200 [12] $1.8x10^3$ - $4.7x10^3$ [15]
Fecal coliforms	CFU/mL	$1x10^3$	$4x10^3$ [B]	0.52 - 109 [12] 0.7 - 23 [11] 1.7 - 316 [8] 100 [C] [14]
Salmonella spp.		+	+	$1x10^3$ CFU/mL [14]
Staphylococcus aureus		-	+ [B]	
Vibrio cholerae		+	+	

[A]The results were obtained from the sample taken at October 8, 2012.
[B]The results were obtained from the sample taken at March 11, 2013.
[C]Tentative guideline value [14].
+Presence detected in presumptive test.
- Not detected in presumptive test.

The detection of *Salmonella* spp., *Staphylococcus aureus* and *Vibrio cholerae* in the analyzed samples suggests the possibility of crossed contamination of the fish tissues and other edible parts, and even a possible transmission to consumers. For this reason, further work is needed to establish a Mexican microbiological quality standard for aquaculture water.

Conclusions and perspectives

The characterization of the effluents of the studied aquaculture farm indicates that the biological treatment constitutes an economical and feasible way to meet the guidelines for RAS. It has been demonstrated that biofilters are an environment-friendly alternative for the removal of TSS, BOD_5 and TKN from aquacultural effluents without excessive surface requirements. Consequently, a two-stage biofiltration process involving nitrification and denitrification seems well-suited for this purpose. This process must also assure the removal of the microbiological pollutants found (*i.e.*, fecal coliforms, *Salmonella* spp., *Staphylococcus aureus* and *Vibrio cholerae*) in order to avoid the contamination of the cultured fish.

The full removal of nitrogen involves heterotrophic microorganisms, as dissimilatory reduction of nitrates is coupled to the oxidation of organic matter. However, the levels measured for TOC, COD and BOD_5 in the samples point out that it is necessary to use an organic support in the denitrifying stage of the process. A lignocellulosic matrix could

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provide the organic carbon needed to this end. At present, we are evaluating different materials (wood chips, nutshells and barley) as biodegradable carbon sources.

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Capítulo 6

Uso de Carbón Activado Granular (CAG) en un biofiltro para el tratamiento de efluentes acuícolas

Uno de los materiales más usados en los sistemas de biofiltración es el carbón activado granular (CAG). En este trabajo se evaluó la capacidad de adsorción de un CAG de origen vegetal tanto de una molécula modelo (el azul de metileno, AM) como de los contaminantes presentes en efluentes acuícolas. Para evaluar la remoción del AM, se realizaron pruebas estáticas por lote, cuyos resultados se ajustaron al modelo de Langmuir. Así mismo, se realizaron pruebas dinámicas para estudiar la cinética de adsorción del AM; los resultados de estas pruebas se ajustaron a un modelo de pseudo segundo orden. Para evaluar la capacidad de remoción de los contaminantes de un efluente acuícola, se hicieron ensayos continuos en una columna empacada con CAG. En estos ensayos se eliminó alrededor de un 60% de la DQO, UV_{254}, $N\text{-}NH_4^+$ y P-total del efluente, mientras que se observó una capacidad de eliminación decimal de coliformes totales de 0.68. Sin embargo, dado que sólo se eliminó el 26% del NTK, es necesario implementar un proceso biológico para la remoción total de este contaminante.

Uso de Carbón Activado Granular (CAG) en un biofiltro para el tratamiento de efluentes acuícolas

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Resumen (BBR)

En este trabajo se caracterizó un carbón activado granular (CAG) en términos de su capacidad de adsorción tanto de una molécula modelo (el azul de metileno, AM) como de los contaminantes presentes en efluentes acuícolas. Para evaluar la remoción del AM, primero se realizaron pruebas estáticas por lote, cuyos resultados se ajustaron al modelo de Langmuir y permitieron estimar una capacidad de adsorción máxima de 67.57 mg AM/g CAG. Así mismo, se realizaron pruebas dinámicas para estudiar la cinética de adsorción del AM; los resultados de estas pruebas se ajustaron adecuadamente a un modelo de pseudo segundo orden y arrojaron capacidades de adsorción y constantes cinéticas comprendidas entre 4.21–14.81 mg AM/g CAG y 0.046–0.421 g CAG/mg AM·h, respectivamente. Para estudiar la capacidad de remoción de los contaminantes de un efluente acuícola, se hicieron ensayos continuos en una columna empacada. En estos ensayos se pudo eliminar alrededor de un 60% de la DQO, UV_{254}, $N\text{-}NH_4^+$ y P-total del efluente acuícola, mientras que se observó una capacidad de eliminación decimal de coliformes totales de 0.68. Sin embargo, dado que sólo se eliminó el 26% del N total Kjeldhal, se advirtió que es necesario implementar un proceso biológico para la remoción total de este contaminante.

Palabras clave: Acuacultura, adsorción, azul de metileno, biofiltro, modelo de Langmuir

Abstract (BBR)

In this work, we characterized the adsorptive properties of a granular activated carbon (GAC) for both methylene blue (MB, a model compound) and the pollutants of aquacultural wastewater. First, the removal of MB was studied in static tests, which allowed the maximal adsorption capacity to be estimated at 67.57 mg MB/g GAC by fitting of Langmuir equation. Dynamic tests were also carried out, and their results could be properly depicted by a pseudo-second order kinetic model. From these tests, equilibrium adsorption capacities and kinetics constants were measured as 4.21–14.81 mg MB/g GAC and 0.046–0.421 g GAC/mg MB·h, respectively. Finally, continuous adsorption tests were conducted in a fixed-bed GAC column fed with aquacultural wastewater. COD, UV_{254}, $N\text{-}NH_4^+$ and total P were removed at substantial levels (about 60%), and an important decimal elimination capacity of total coliforms (0.68) could be measured. However, since only 26% of total Kjeldhal N could be eliminated by adsorption, further biological treatment of the wastewater was deemed necessary for the full removal of this pollutant.

Key words: Aquaculture, adsorption, biofilter, Langmuir equation, methylene blue

INTRODUCCIÓN

En México, el desarrollo de la acuacultura es muy importante debido a que permite obtener, a bajo precio, alimentos con altos contenidos de proteínas y lípidos, así como de una amplia variedad de nutrientes. También tiene gran relevancia económica, ya que es el sector de producción animal que más rápido se expande en el mundo [1].

Por otra parte, la acuacultura tiene un impacto ambiental considerable, ya que ocupa el segundo lugar en cuanto a demanda de agua en México (42%) [2]. En consecuencia, es una actividad que descarga grandes volúmenes de aguas residuales, que se caracterizan por la presencia de alimento sin ingerir, metabolitos y aditivos destinados a aumentar la producción, entre otros contaminantes. Si estos efluentes se descargan sin recibir un tratamiento adecuado, pueden aportar sólidos suspendidos, materia orgánica, nutrientes (como amonio y fosfatos) y microorganismos patógenos a los cuerpos receptores, y afectar así la calidad del agua [1].

Una solución para la eliminación o la reducción de la concentración de estos compuestos es el uso de Carbón Activado Granular (CAG), que por ser un adsorbente

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eficiente y de bajo costo es el más empleado para el tratamiento de aguas residuales [3]. La capacidad de adsorción del carbón activado se debe a que es altamente poroso y posee un área superficial elevada [4]. Esta capacidad de adsorción depende del tamaño medio de las partículas de carbón, del material a partir del cual se preparó y del pH de la solución acuosa, así como de la naturaleza química del adsorbato.

Para determinar la capacidad de adsorción de un carbón activado en medio líquido, es frecuente llevar a cabo ensayos con yodo o con azul de metileno (AM). Los primeros permiten estimar el número de yodo, que es una medida de la capacidad de adsorción de solutos con bajo peso molecular, por lo general inorgánicos, y por lo tanto ligada a la microporosidad. En cambio, los ensayos con AM sirven para determinar la capacidad de adsorción de compuestos orgánicos con peso molecular semejante al de este pigmento (319.9 g/mol), la cual a su vez está definida por la mesoporosidad del carbón [5].

Los biofiltros de CAG son una alternativa interesante para el tratamiento de efluentes acuícolas, ya que ofrecen soporte a poblaciones microbianas (*i.e.*, capaces de biodegradar o nitrificar), al tiempo que remueven contaminantes por adsorción. Así, el objetivo de este estudio fue la caracterización del CAG que se utilizará en un biofiltro para el tratamiento de efluentes acuícolas. Para ello, se llevaron a cabo ensayos de adsorción por lote (estáticos y dinámicos) con AM, así como ensayos continuos en una columna empacada alimentada con agua residual de la industria acuícola.

MATERIAL Y MÉTODOS

Carbón activado granular

El CAG empleado es de origen vegetal (elaborado a partir de cáscara de coco; Filtratec, Pachuca, Hgo.), con micro a mesoporos (5-50 nm). La Fig. 1 muestra una fotografía del CAG obtenida con un microscopio electrónico de barrido (Jeol JSM-6300, Japón). Una muestra se recubrió con oro en una evaporadora (Dentum Bacuum, DESK II, E.U.A.) durante 2 minutos, a una presión de 20 militorrers. Posteriormente, la muestra se colocó en un soporte y se introdujo al microscopio.

Previamente a las pruebas de adsorción, el carbón se humedeció en agua destilada y se mantuvo en agitación por 12 h, tras las cuales se recuperó por filtración. Para conocer el peso seco de CAG introducido a las pruebas de adsorción, se determinó su humedad en una muestra que se secó a 105°C por 24 h.

Pruebas de adsorción por lote

Las pruebas se llevaron a cabo en vasos de precipitados de 500 mL a los que se agregaron 200 mL de solución de AM (10^{-5} M). El pH de la solución de AM se ajustó previamente a 7 con HCl 0.1 M, debido a que el pH modifica los resultados de las pruebas de adsorción [4]. A cada vaso se agregaron diferentes cantidades de CAG. El contenido de cada vaso se mantuvo en agitación (aprox. 100 rpm) con una barra magnética.

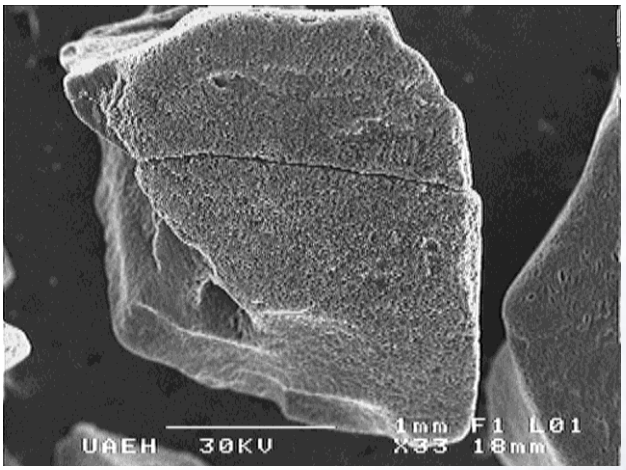

Fig. 1. Fotografía del CAG en microscopía electrónica de barrido.

Para las pruebas estáticas, se tomaron muestras una vez alcanzado el equilibrio (*i.e.*, tras 24 h de tiempo de contacto). Para las pruebas dinámicas, el muestreo se realizó a intervalos de tiempo regulares. Cada muestra se centrifugó por 10 minutos a 10,000 rpm en una centrífuga MiniSpin® (Eppendorf, Alemania) para separar el CAG. La absorbancia a 655 nm del sobrenadante se midió de inmediato en un espectrofotómetro Genesys 10 vis® (Thermoscientific, E.U.A.) y se comparó contra una curva estándar para calcular la concentración de AM.

Análisis de las pruebas estáticas de adsorción. Los resultados de las pruebas estáticas se ajustaron a la ecuación de Langmuir (1):

$$q_e = \frac{q_{max}\, b\, C_e}{1 + b\, C_e} \qquad (1)$$

donde q_e es la capacidad de adsorción (mg de AM por g de CAG) en el equilibrio, C_e es la concentración de AM (mg/L) en el equilibrio y q_{max} y b son constantes empíricas. Éstas se determinaron por linearización de (1):

$$\frac{C_e}{q_e} = \frac{1}{b q_{max}} + \frac{C_e}{q_{max}} \qquad (2)$$

Así, al graficar (C_e/q_e) contra C_e se ajustó por regresión lineal una ecuación cuyos valores de ordenada al origen y pendiente permitieron obtener los valores de q_{max} y b.

Análisis de las pruebas dinámicas de adsorción. Se usó un modelo de pseudo segundo orden [6] para describir las cinéticas de adsorción (3), cuya forma integrada (con los límites t = 0 a t = t y q_t = 0 a $q_t = q_t$) está dada por (4):

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$$\frac{dq_t}{dt} = k(q_e - q_t)^2 \quad (3)$$

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + kt \quad (4)$$

donde q_t es la capacidad de adsorción (en mg AM por g CAG) a un t dado, q_e es la capacidad de adsorción en el equilibrio y k (en g CAG por mg AM y por h) es la constante cinética de pseudo segundo orden. Por linearización de (4) se obtiene la siguiente ecuación:

$$\frac{t}{q_t} = \frac{1}{k\,q_e^2} + \frac{1}{q_e}t \quad (5)$$

Así, k y q_e se obtuvieron de la pendiente y de la ordenada al origen de la línea obtenida al graficar (t/qt) *vs*. t [6].

Pruebas continuas de adsorción

Se usó un contactor de lecho fijo de 360 cm^3 de volumen total y 346.4 cm^3 de volumen de lecho empacado con CAG (Fig. 2). La columna se alimentó por la parte superior con 5.55 mL/min de efluente acuícola con una bomba peristáltica (Masterflex®, E.U.A.). Estas condiciones equivalen a un tiempo de residencia de lecho vacío (TRLV) de 62.4 min.

Muestreo de efluentes acuícolas. El muestreo se llevó a cabo el 1 de junio de 2013. Se obtuvo una muestra compuesta de alrededor 3.5 L de un estanque de la Granja Integral de Policultivo (Tezontepec de Aldama, Hgo.). Se tomaron 4 muestras individuales en diferentes puntos del estanque, dedicado principalmente a la producción de *Cyprinus* spp., que luego se mezclaron para obtener una muestra compuesta. Una parte de la muestra se almacenó en recipientes estériles de un litro para llevar a cabo los análisis microbiológicos. Las muestras se transportaron al laboratorio y se preservaron a 4°C hasta su análisis. Las determinaciones microbiológicas se hicieron el mismo día del muestreo.

Análisis de efluentes acuícolas. El análisis del agua de entrada y salida de la columna se llevó a cabo según los métodos estándar de la APHA [7]. Los sólidos suspendidos totales (SST) se midieron gravimétricamente a 105 ± 5°C (método 2540-D). La demanda química de oxígeno (DQO) se analizó después de digestión de las muestras por medición espectrofotométrica (600 nm) de la disminución en la concentración de Cr(VI) (método 5520). La UV_{254} se midió en una celda de cuarzo de 10 mm de trayecto óptico, en un espectrofotómetro Lambda 40 UV-Vis (Perkin Elmer, E.U.A.). Para medir el nitrógeno total Kjeldahl (NTK), las muestras se sometieron a digestión a 400°C y se destilaron (Gerhardt® Vapodest 20, Alemania) para transformar el nitrógeno orgánico en NH_4^+, el cual se determinó posteriormente por valoración con HCl 0.01N (método 4500-B). El N-NH_4^+ se analizó por el método del fenato (4500-NH_3). Para medir la concentración de P total, se usó el método del ácido ascórbico (4500-P F). La cantidad de coliformes totales se determinó según el método de vertido en placa sobre agar selectivo cromogénico (Hi Chrome™) con base de agar soya tripticasa. Después de 24 h de incubación a 35 ± 2°C, se contaron las colonias de color rojo [8]. Todas las determinaciones se hicieron por duplicado.

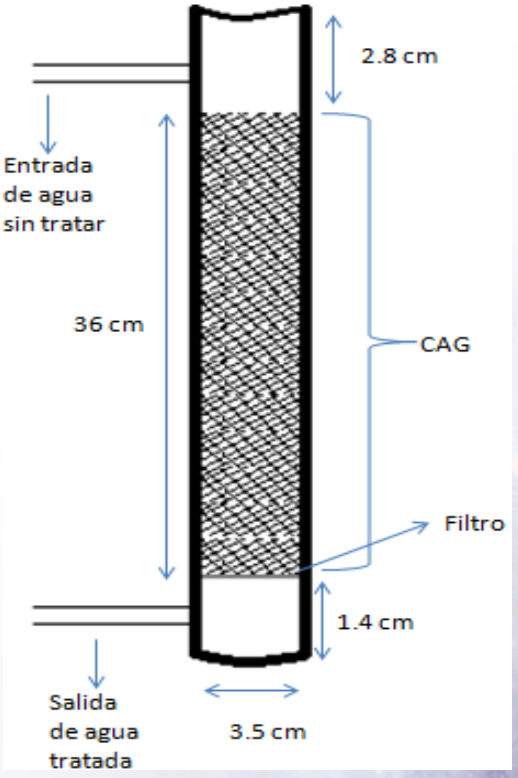

Fig. 2. Columna usada en las pruebas continuas de adsorción.

Modelado de la remoción de contaminantes en la columna de adsorción. Los resultados de las pruebas continuas se ajustaron a un modelo de decaimiento exponencial (SigmaPlot 12.0, Systat Software Inc., E.U.A.):

$$\frac{C}{C_0} = ae^{-bt} \quad (6)$$

donde C es la concentración de cada contaminante a la salida de la columna en un instante dado y C_0 es la concentración medida a la entrada de la columna, mientras que a y b son parámetros empíricos.

Cálculo de la capacidad de eliminación decimal (CED). La CED de los coliformes totales del GAC se calculó a partir de las concentraciones a la entrada (C_0) y a la salida (C) de la columna [9]:

$$\mathrm{CED} = \log_{10}\frac{C_0}{C} \quad (7)$$

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RESULTADOS Y DISCUSIÓN

Análisis de las pruebas de adsorción por lote

Análisis de las pruebas estáticas. Los resultados se ajustaron adecuadamente a la ecuación de Langmuir, ya que al graficar (C_e/q_e) contra C_e se obtuvo un buen ajuste a una línea recta (r=0.98; gráfico no mostrado).

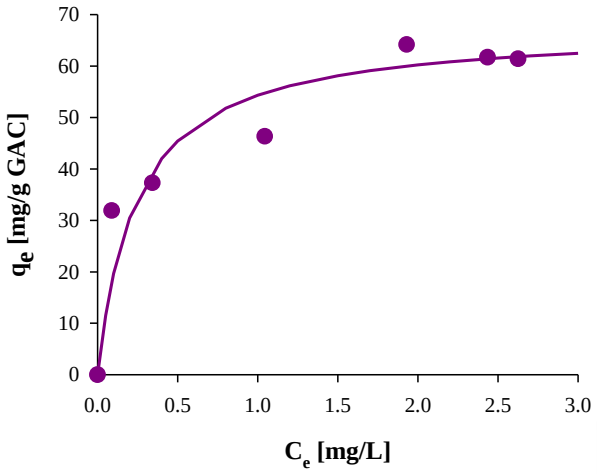

Fig. 3. Isoterma de adsorción de azul de metileno al CAG. Los puntos representan los datos experimentales y la línea continua, los datos obtenidos por simulación de la ecuación de Langmuir.

Con la ecuación de esta línea recta se obtuvieron los valores de q_{max} (67.57 mg AM/g GAC) y b (4.11 L/mg). La Fig. 3 muestra la isoterma correspondiente a la adsorción de AM al CAG, así como los resultados de la simulación con los parámetros determinados. La literatura reporta por lo general mayores capacidades de adsorción de AM; por ejemplo, para CAG obtenidos a partir de mijo, bagazo de caña y sorgo, se reportaron q_{max} de 390, 502 y 662 mg AM/g CAG [5]. Es posible que en el presente estudio las pruebas estáticas de adsorción hayan subestimado la q_{max} real del CAG, ya que sólo se emplearon concentraciones reducidas del colorante y éste es un factor que modifica los valores de las constantes de Langmuir [3].

Análisis de las pruebas dinámicas. En la Tabla 1 se muestra un resumen de las constantes del modelo de pseudo segundo orden que se calcularon para los ensayos de adsorción, así como los coeficientes de correlación que se obtuvieron al linearizar los datos según (5).

En términos generales, a mayor masa de CAG el ajuste al modelo cinético de pseudo segundo orden es mejor. No se observó ninguna tendencia en los valores de k; sin embargo, se encontró que el valor de la capacidad de adsorción en el equilibrio (q_e) aumenta al introducir menores cantidades de CAG en los ensayos dinámicos, lo cual confirma lo reportado por otros autores [10].

Tabla 1. Resumen de constantes cinéticas calculadas.

Masa CAG seco [mg]	k [g CAG/mg·h]	q_e [mg /g CAG]	r
53	0.046	14.81	0.96
61	0.321	7.08	0.98
86	0.076	9.74	0.97
117	0.101	7.21	0.97
122	0.294	4.34	0.98
142	0.247	5.90	0.98
173	0.164	5.18	0.97
226	0.421	4.21	0.99

El CAG analizado tiene una capacidad de adsorción menor a la reportada en la bibliografía (218.8 – 309.1 mg AM/g CAG) [4]. Al igual que en los estudios estáticos, la q_e pudo haber sido subestimada al utilizar una baja concentración de AM en los ensayos. Sin embargo, las constantes cinéticas determinadas en el presente estudio son sustancialmente mayores a las reportadas previamente ($1.06x10^{-4}$–$1.49x10^{-4}$ g CAG/mg AM·h) [4].

En la Fig. 4 se muestran los resultados de los ensayos dinámicos realizados con diferentes cantidades de CAG, así como los resultados de simulación del modelo de pseudo-segundo orden.

Análisis de las pruebas continuas de adsorción

Calidad del agua de alimentación. La Tabla 2 resume las características del efluente acuícola que se alimentó a la columna de adsorción. Los valores obtenidos de DQO, NTK, P-total y coliformes totales son similares a los obtenidos previamente en el mismo sitio de muestreo: 208.2, 400.5 y 11.9 mg/L y 1.6 $x10^3$ UFC/mL respectivamente [11]. La presencia de DQO y NTK en altas concentraciones se asocia a la presencia de materia fecal producida por los peces, alimento no ingerido e incluso a animales muertos localizados en el fondo de los estanques, mientras que la presencia de estos contaminantes microbiológicos se ha relacionado con la densidad de peces en el estanque y con la estación del año [12]. Aunque el efluente cumple con los límites máximos permisibles (LMP) establecidos por la NOM-001-SEMARNAT-1996 para los SST (150 mg/L) y el P total (20 mg/L) de descargas a ríos con calidad apta para riego agrícola, no ocurre lo mismo si se considera el NTK, cuyo LMP es de 40 mg/L.

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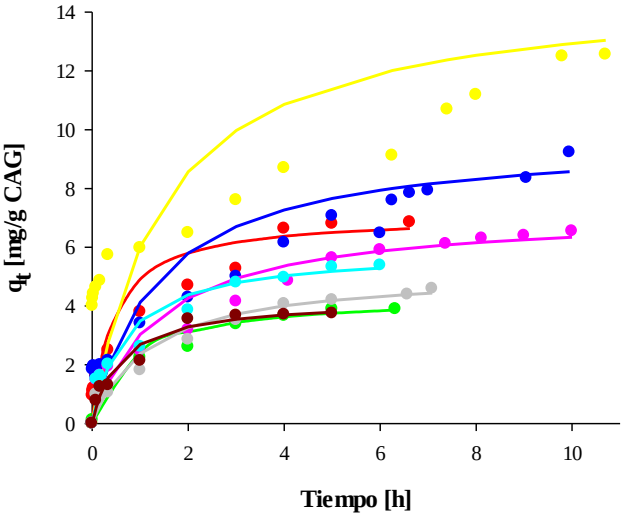

Fig. 4. Capacidad de adsorción de azul de metileno en función de la cantidad de CAG (mg) usado en las pruebas: (●)53; (●)61; (●)86; (●)117; (●)122; (●)142; (●)173; (●)226. Los puntos representan los datos experimentales y las líneas continuas, los datos obtenidos por simulación del modelo de pseudo segundo orden.

Tabla 2. Calidad del agua de alimentación usada en las pruebas continuas de adsorción.

Parámetro	Valor medido
pH	8.4
SST [mg/L]	20.5
DQO [mg/L]	226.7
UV_{254}	0.477
NTK [mg/L]	406.0
$N\text{-}NH_4^+$ [mg/L]	0.93
P-total [mg/L]	11.4
Coliformes totales [UFC/mL]	$1{\cdot}10^3$

Dado que el contenido de nutrientes (N y P) es alto, estos efluentes deben recibir algún tratamiento antes de ser descargados; para ello, el tratamiento biológico (*e.g.*, por humedales o biofiltros) es la mejor opción. Sin embargo, se debe tener en cuenta que los efluentes acuícolas analizados presentan concentraciones de nitrógeno desproporcionadamente elevadas en relación con el contenido de carbono; para ello se debe recurrir a una fuente adicional de carbono que ayude a aumentar la relación C:N [1].

Remoción de contaminantes. La Fig. 5 muestra las concentraciones de los contaminantes analizados en el agua de salida de la columna al cabo de 1-4 días, así como la CED de los coliformes totales. En la figura no se muestran las concentraciones que se midieron para los SST, ya que las partículas finas de CAG que se desprendieron de la columna durante los tres primeros días interfirieron con los análisis.

El buen ajuste (r>0.92) de las cinéticas al modelo de decaimiento exponencial indica que la velocidad de remoción de DQO, UV_{254}, NTK, $N\text{-}NH_4^+$ y P-total fue inicialmente rápida y luego disminuyó conforme transcurrieron los días.

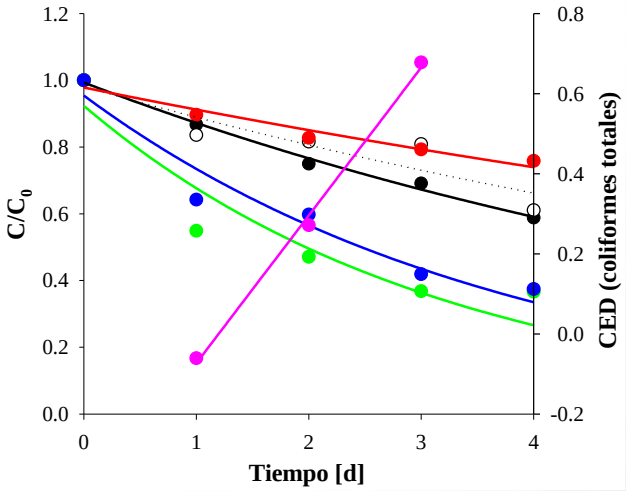

Fig. 5. Remoción de contaminantes en los ensayos continuos de adsorción. Los puntos representan los datos experimentales y las líneas, el ajuste a un modelo de decaimiento exponencial con dos parámetros.
(●) DQO; (○) UV_{254}; (●) NTK; (●) $N\text{-}NH_4^+$; (●) P-total; (●) CED (coliformes totales).

Una posible explicación de este comportamiento es que con el paso de los días se fue desarrollando una biopelícula que modificó el mecanismo de remoción de los contaminantes. Así, mientras luego del arranque de la columna estos se removieron principalmente por adsorción sobre el CAG, al transcurrir el tiempo los microorganismos empezaron a competir con los contaminantes por sitios de adhesión. El CAG es un excelente soporte microbiano, ya que debido a su superficie irregular y a su elevada porosidad, posee numerosos sitios para que los microorganismos se adhieran [13]. Por lo anterior, se prefiere el CAG a otros materiales de empaque no adsorbentes, como la arena o la antracita, y es el relleno más utilizado en biofiltros [14].

Las concentraciones de DQO y UV_{254} mostraron tendencias similares de remoción en la columna, y una eliminación máxima de 59 y 61%, respectivamente, al cabo de cuatro días. La UV_{254} está asociada a grupos aromáticos, y se le usa como un indicador de la concentración de sustancias húmicas [15] y de la fracción refractaria de la materia orgánica natural. A pesar de que sólo representa el carácter aromático y la hidrofobicidad de ésta, se le considera un sustituto potencial del análisis de carbono orgánico total (COT) [15]. Nuestro trabajo parece confirmar la correlación que existe entre la UV_{254} y la materia orgánica, en este caso determinada como DQO (r=0.84).

El menor nivel de remoción se observó con el NTK, ya que al término del estudio el agua de salida de la columna contenía aún el 74% de la concentración inicial en el agua de entrada. En un estudio que estimó la

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adsorción del NTK y la DQO del agua residual de un rastro sobre CAG [16], se estimaron capacidades de adsorción máximas de 48 y 915 mg/g, respectivamente. Esta reducida adsorbabilidad del NTK sobre el CAG concuerda con la baja remoción observada en la columna. Dado que la concentración del NTK es aún muy alta a la salida de la columna (*i.e.*, 308 mg N/L), es necesario poner en marcha un proceso biológico que permita su remoción total y posteriormente su descarga o reúso.

El N-NH_4^+ y el P-total se removieron con tendencias semejantes y con eficiencias elevadas (63.4 y 62.6%, respectivamente, al cabo de 4 días). En un estudio que buscó comparar la capacidad de adsorción de ortofosfatos sobre tres materiales (CAG, zeolita y ceramisita), se concluyó que el CAG era la mejor opción, puesto que la concentración inicial disminuyó en un 83% en pruebas por lote estáticas [17].

En cuanto a la remoción microbiana, la CED del CAG fue negativa el primer día, pero aumentó hasta alcanzar un valor de 0.68 al término del tercer día (equivalente a una eliminación del 79% de los coliformes totales presentes en el agua de entrada). Éste es un valor de CED superior al medido en un estudio previo (0.4) y correspondiente sólo a la eliminación de *Escherichia coli* en un filtro de CAG nuevo y con un tiempo de contacto de 12 minutos [9]. En ese mismo estudio, los autores mencionan que el CAG presenta comúnmente CED de 0.5–1.1 log para *E. coli*, mientras que las remociones suelen ser mayores para quistes de *Cryptosporidium parvum* (1.1–2.7 log) y para *Giardia lamblia* (2.0 log). Aunque estos resultados permiten considerar a los filtros de CAG como una barrera contra protistas patógenos, cuya eficiencia aumenta conforme progresa la colonización microbiana [18], no ocurre lo mismo contra bacteriófagos [9]. Los mecanismos por los cuales los microorganismos se adhieren al CAG son fuerzas electrostáticas y de van der Waals a cortas y largas distancias, respectivamente [9].

CONCLUSIÓN

En pruebas de adsorción por lote (estáticas y dinámicas), el CAG mostró una capacidad de adsorción menor a la reportada en la bibliografía para una molécula modelo, el azul de metileno. Sin embargo, en pruebas continuas en una columna de adsorción alimentada con un efluente acuícola, removió significativamente el contenido de N-NH_4^+, P-total y coliformes totales. Aunque la carga contaminante del efluente se redujo de modo importante, aún es necesario implementar un sistema de tratamiento biológico para la remoción total del NTK del agua que permita su descarga al medio acuático o bien su reúso en el proceso productivo.

Reconocimientos

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Capítulo 7

Evaluation of Natural Materials as Exogenous Carbon Sources for Biological Treatment of Low Carbon-to-Nitrogen Wastewater

La eutrofización es un problema de contaminación del agua debido al enriquecimiento excesivo de nutrientes (nitrógeno y fósforo), que afecta los ecosistemas. Para mitigar la contaminación originada por compuestos nitrogenados es necesaria la implementación de tratamientos biológicos, en cuyo diseño es crucial la relación carbono:nitrógeno. Un valor bajo de esta relación en aguas residuales, como ocurre con los efluentes acuícolas de la Granja Integral de Policultivo de Tezontepec de Aldama, Hgo., requiere la adición de fuentes exógenas de carbono.

Para seleccionar una fuente exógena de carbono, se evaluaron materiales naturales y económicos (aserrín, granos de cebada y cáscaras de cacahuate). Los materiales se sometieron a pruebas de lixiviación, en las que se midió la cantidad de materia orgánica, compuestos nitrogenados y fósforo total liberados al medio acuoso. Además, las características de las materias primas y la materia orgánica lixiviada se estudiaron mediante espectroscopía de infrarrojo y análisis elemental. Los resultados indicaron que el aserrín y las cáscaras de cacahuate son fuentes adecuadas de carbono, ya que liberan materia orgánica y una baja cantidad de nutrientes. En particular, el aserrín libera carbono de manera constante, por lo que no necesita ser reemplazado con frecuencia de los reactores biológicos; además, no libera fósforo al medio. En consecuencia, es un material adecuado para compensar limitaciones de carbono en procesos biológicos de tratamiento del agua.

Evaluation of natural materials as exogenous carbon sources for biological treatment of low carbon-to-nitrogen wastewater

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1. Introduction

Anthropogenic eutrophication is a major water pollution problem worldwide. Over-enrichment of nutrients (nitrogen and phosphorus) increases the production of biomass in aquatic systems, thereby impairing the water quality and threatening the natural balance of these ecosystems. Agricultural and urban runoff, livestock operations, aquaculture, industry (e.g., food processing facilities and pulp and paper mills), sewage treatment plants and fossil fuel combustion are the major sources of nutrient pollution [1].

The mitigation of nitrogenous pollution relies on biological treatments based on the well-known route ammonification-autotrophic nitrification-heterotrophic denitrification. In these processes, the carbon-to-nitrogen (C:N) ratio is a key design parameter. Although a rough 10:1 ratio (measured as COD/TKN) is frequently recommended, some authors suggest values as high as 20:1 or 30:1 [2]. Besides, at high C:N ratios heterotrophic bacteria can out-compete nitrifying microorganisms [3]. In contrast, low C:N ratios limit denitrification and can cause the accumulation of NO_2^- in full nitrogen removal processes [4].

Many of the aforementioned pollution sources generate effluents with disproportionately high contents of nitrogen. For instance, wastewater from optoelectronics industries is particularly enriched in organic nitrogen because ethanolamine and tetra-methyl ammonium hydroxide are commonly used in the manufacturing process [5]. Intensive aquaculture systems tend to generate effluents enriched in NH_4^+ [6-7], as well as petrochemical, pharmaceutical, fertilizer and food industries [8]. Stainless steel manufacturing processes generate wastewater with nitrate concentrations ranging from 500 to 1000 mgN-NO_3^-/L [9], while in agriculturally-impacted groundwater a range of 1-2 mgN-NO_3^-/L is expected [10]. In the two last cases, hardly any organic matter is to be found.

Any unbalanced C:N wastewater requires the addition of exogenous carbon sources. A wide variety of compounds have been employed for this purpose, such as sugars, organic acids, alcohols and oils [11], though methanol appears to be the most commonly employed [9]. Recently, solid materials have received more attention, and consequently cornstalks [11], wood by-products (e.g., sawdust and woodchips) [12], wheat straw [13], microbial cellulose [14], compost [15], and starch-based biodegradable polymers [16] –among other substrates– have also been evaluated as external carbon donors.

For selecting a carbon source, several aspects must be considered, such as its cost, resulting denitrification rate, handling safety and potential release of toxic compounds. The costs of the carbon donor and the sludge management are key, as they account for more than 50% of the overall wastewater treatment cost [9]. Another important aspect of the carbon source to take into account is its pollution swapping potential, which is the increase in one pollutant concentration as a result of an action implemented to remove another pollutant [17].

We evaluated economical and natural materials (wood chips, feed barley grains and peanut shells) as potential carbon sources for low carbon-to-nitrogen wastewater. In leaching tests, we measured the amount of organic matter released from these materials in aqueous media, as well as their pollution swapping potential in terms of release of nitrogenous compounds (total Kjeldhal nitrogen, N-NH_4^+, NO_2^- and NO_3^-) and total phosphorous. Besides,

the characteristics of the raw materials and the leached organic matter were studied by infrared spectroscopy.

2. Materials and methods

2.1 Natural materials. Wood chips, feed barley and peanut shells were studied as potential carbon sources. Wood chips were obtained from untreated pine (*Pinus sylvestris*). 85.5% of the woodchips was retained over a 2 mm mesh sieve, while 10.4% passed through a 2 mm mesh sieve but was retained over a 1 mm mesh sieve. The remaining fraction (4.1%) passed through a 1 mm mesh sieve but was retained over a 0.6 mm mesh sieve. Barley (*Hordeum vulgare* L.) grains (0.6 ± 0.05 cm) and peanut shells (3.2 ± 0.47 cm) were obtained from Apan and Temascalapa, Mexico, respectively. All foreign matter (as stones and dirt) and damaged grains were removed from barley by hand.

Elemental composition analysis and Fourier Transform Infrared (FT-IR) analyses were made on 1.5 – 3.0 mg samples of the natural materials previously ground to a homogeneous fine powder and dried at 105°C for 24 h. A 2400 Series II CHNS Elemental Analyzer and a Spectrum GX FT-IR spectrometer (both from Perkin–Elmer™, Waltham, USA) were used, respectively. The total carbon and hydrogen contents were 50.56 and 6.36% for wood chips, 48.01 and 5.31% for barley and 44.49 and 6.47% for peanut shells, respectively. Nitrogen was only detected in barley samples, where it accounted for 0.97%. The IR spectra were obtained from KBr pellets (1:100 weight ratio of sample/KBr). The spectrometer was set to scan from 4000 to 400 cm^{-1}.

2.2 Batch leaching tests. Leaching tests were performed in batch mode at a solid-to-liquid ratio of 50 g/1 L. The materials were washed with distilled water and air dried. Samples of the natural materials were added separately to 1 L of distilled water in glass flasks. The flasks were purged for 10 min with nitrogen, sealed and then placed under agitation on an orbital shaker (Polyscience™, USA) at 120 rpm and at room temperature for 50 days. Periodically, 100 mL-samples of the supernatants were taken from each flask and maintained at 4°C until analysis.

After 50 days, the supernatants were completely withdrawn from the flasks, filtered and separated in about 1 mL-portions to be lyophilized by continuous freeze drying (Freeze Dry System, Freezone 4.5, Labconco™, Kansas City, USA) at –50°C and $133x10^{-3}$ mBar.

2.3 Chemical analysis of leachates. Liquid samples of the eluates from the leaching tests were analyzed for chemical oxygen demand (COD), total organic carbon (TOC), biological oxygen demand after 5 days (BOD_5), total Kjeldahl nitrogen (TKN), $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and total phosphorous (TP). Except where indicated otherwise, the analyses were made according to the Standard Methods [18]. COD was analyzed spectrophotometrically at 600 nm after digestion of the samples with $K_2Cr_2O_7/H_2SO_4$ (method 5520). TOC was measured with an infrared analyzer (Shimadzu™, Japan). BOD_5 was determined in the OxiTop™ measurement system (WTW, Germany). For measuring TKN, the samples were digested at 400°C and distilled in a Gerhardt® Vapodest 20 (Germany) unit to transform organic nitrogen into ammonium ions, which were further assessed by titration with 0.01N HCl. $N\text{-}NH_4^+$ was assessed by the phenate method (4500-NH_3). The 4500-NO_2^- and 4500-P F methods were

used for measuring $N\text{-}NO_2^-$ and TP, respectively. Finally, the content of $N\text{-}NO_3^-$ was determined by the phenoldisulfonic acid method [19].

Lyophilized leachates were analyzed for elemental composition and FT-IR spectroscopy as for raw natural materials (cf. 2.1 section).

3. Results and discussion

3.1 FT-IR analysis of the raw natural materials. FT-IR spectra are associated to the transitions between different levels of vibrational energy. These levels correspond to vibrations (e.g., bendings of bonds and other complex movements of the molecules). Table 1 summarizes the main band assignments of the FT-IR spectra obtained.

Table 1. Main characteristics of the FT-IR spectra of the raw natural materials

Material	$\bar{\nu}$ **(cm^{-1})**	**Assignment**
Wood chips	3425	$\upsilon_{O\text{-}H}$
	2923	$\upsilon_{C\text{-}H}$
	2854	$\upsilon_{C\text{-}H}$
	1729	$\upsilon_{C=O}$
	1637	$\upsilon_{H\text{-}O\text{-}H}$
	1033	$\upsilon_{P\text{-}O\text{-}C}$
	759	$\upsilon_{P\text{-}C}$
Peanut shells	3431	$\upsilon_{O\text{-}H}$
	2923	$\upsilon_{C\text{-}H}$
	2854	$\upsilon_{C\text{-}H}$
	1631	$\upsilon_{H\text{-}O\text{-}H}$
	1426	$\delta_{C\text{-}H}$
	1034	$\upsilon_{P\text{-}O\text{-}C}$
	754	$\upsilon_{P\text{-}C}$
Barley	3419	$\upsilon_{O\text{-}H}$
	2926	$\upsilon_{C\text{-}H}$
	2855	$\upsilon_{C\text{-}H}$
	1656	$\upsilon_{H\text{-}O\text{-}H}$
	1379	$\upsilon_{N=O}$
	1022	$\upsilon_{P\text{-}O\text{-}C}$
	860	$\delta_{C\text{-}H}$
	767	$\upsilon_{P\text{-}C}$

υ: Stretch vibration; δ: scissor vibration.

For the three materials, bands corresponding to the O-H stretching vibrations were observed in the ≈3400 cm^{-1} region, which can be associated to humidity. C-H stretching bands (≈2900-≈2850 cm^{-1}) associated to asymmetric and symmetric methyl and methylene groups, present profusely in organic compounds, were also detected in the three materials. For wood-like materials, the bands of the second region can be attributed to cellulose bonded to lignin; in fact, this complex accounts for about 45% of the total carbon [20]. In spectra of barley grains, the C-H vibrations (≈2900-≈2850 cm^{-1}) may be related to polysaccharides, mainly starch, found in the pericarp [21] or to the bond of a C-H group to a C-C group. The last case could be attributed to unsaturated lipids of the grains germ [22]. For peanut shells, C-H stretching bands can be associated to carbohydrates.

Other signals of interest were detected for the three materials between 750 y 1000 cm^{-1}, which were assigned to phosphorous inorganic compounds. In the case of agricultural products, this can be explained by the transfer of phosphate fertilizers from soil to the products. Alternatively, the ≈ 1000 cm^{-1} signal can be attributed to the vibration of the phosphodiester group of fatty acids present in barley grains.

In the 1379 cm^{-1} region of the barley spectra, a signal related to nitrates was detected. This is probably due to the assimilation of nitrate-based fertilizers by the plant root system [23].

3.2 Leaching of organic matter to the aqueous media. In leaching tests, submerged plant material releases soluble compounds due to the outbreak of the vacuoles of plant cells by the physical action of water [24]. In this way, if natural materials are used as carbon donors, cell lysis products may lead to swapping pollution.

Figure 1 shows the concentrations of COD measured in the eluates throughout the leaching tests. An appreciable amount of organic matter was released from the three materials in the first two days of testing; it represented about of 45, 28 and 63% of the total COD leached from woodchips, peanut shells and barley grains, respectively, after 50 days. The COD leached from the three materials continued augmenting afterwards, but at slower pace. For peanut shells, the slow phase started after 14 days of testing, rather than after two. This biphasic behavior has been reported for other natural materials, such as wild sugar cane (*Saccharum spontaneous*) [24]. Accordingly, the degradation of submerged organic materials starts by a leaching phase, followed by a hydrolysis phase characterized by the breakdown of macromolecules to simpler compounds.

Since the leaching tests were carried out without an external electron donor (e.g., O_2), no uptake of organic matter was detected. After 50 days, the total COD accumulated in the liquid media was 1063.5, 1198.9 and 2344.2 mg COD/g material for woodchips, peanut shells and barley, respectively.

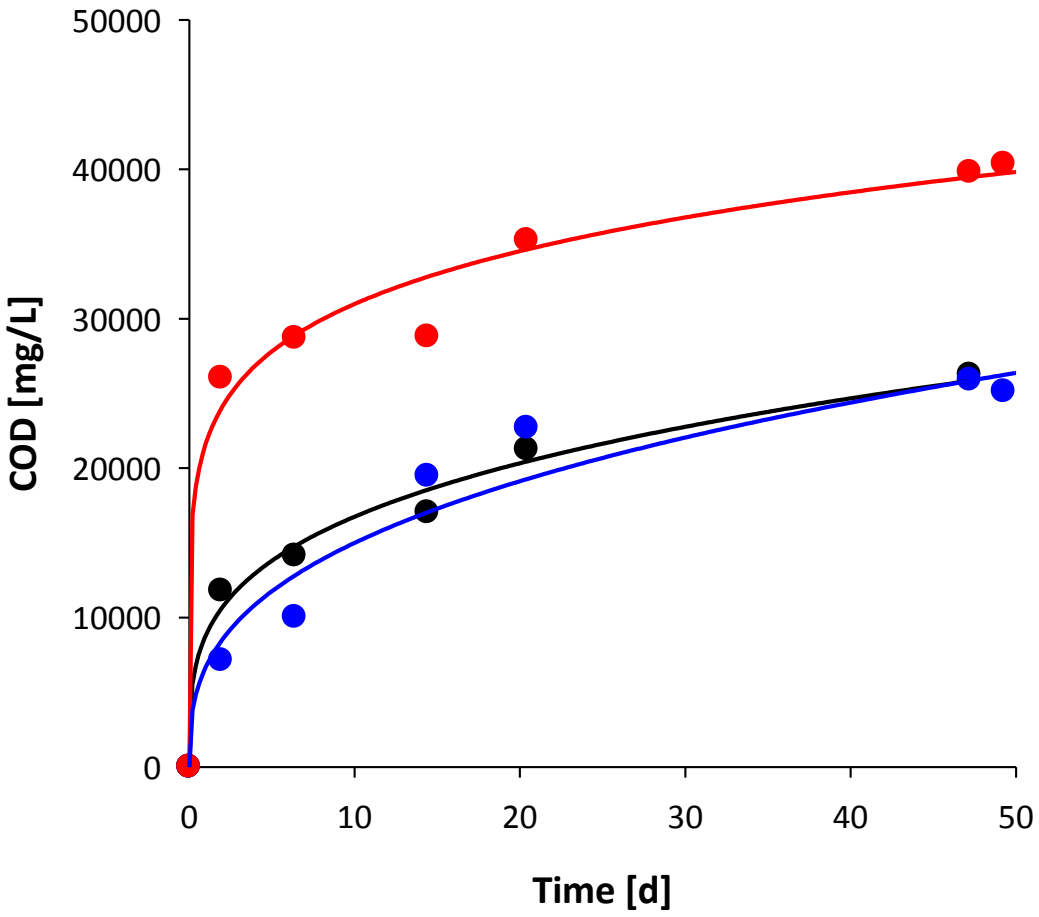

Figure 1. Chemical oxygen demand (COD) released from natural materials.

(●) Woodchips, (●) peanut shells, (●) barley.

BOD_5 was measured only for the samples taken after 30 days of testing (data not shown). For these samples, the calculated BOD_5/COD ratios were 0.15, 0.49 and 0.42%, respectively, thereby indicating that the organic matter leached from woodchips is less readily biodegradable than the organics leached from both peanut shells and barley. A very similar BOD_5/COD ratio (0.14) was reported for an old woodwaste leachate [25]. Wood leachates contain a mixture of hemicellulose, lignins, tannins and fatty acids, among other compounds [25]. The high molecular weight of some of these compounds could explain the low ready biodegradability of the leachates. It has been also reported that wood leachates are toxic due to the presence of tannins, lignins, tropolones, terpenes and lignanes [25]; however, the performance of wood-like materials as carbon donors in biological treatments has been widely experienced [4, 11].

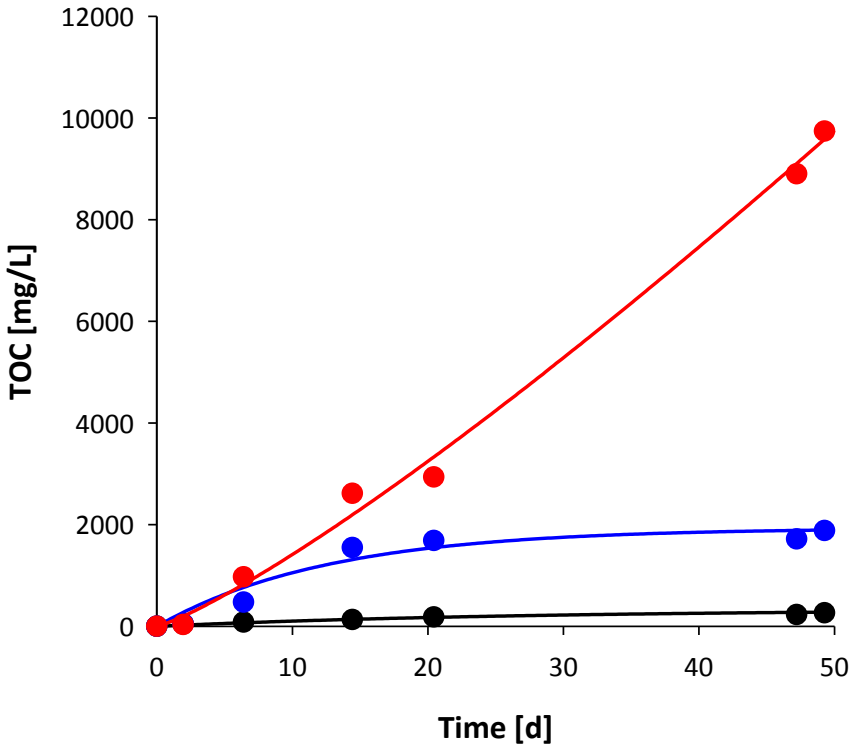

Figure 2. Total Organic Carbon (TOC) released from natural materials.

(●) Woodchips, (●) peanut shells, (●) barley.

If the TOC is considered, barley was the material releasing the highest concentration too (Figure 2). Nonetheless, for this material a linear TOC leaching was observed rather than the aforementioned biphasic behavior. After 50 days of testing, the TOC accumulated was 10.1, 76.1 and 246.5 mg/g of woodchips, peanut shells and barley, respectively. The accumulation of TOC released from woodchips is considerably smaller than the value (i.e., 45 mg/g) previously reported for pine wood chips after only two days of leaching [26], probably due to variations in the wood quality. The TOC released in our assays accounted for only 2, 15.9 and 55.4% of the initial C content measured by the elemental composition analysis in woodchips, peanut shells and barley, respectively. For woodchips with several particle sizes (0.60, 1.18 and 4.75 mm), a prior study [27] had reported a cumulative organic carbon release of 1.1, 0.80 and 0.60% after 7 days, respectively, which is consistent with our results. It is often advocated that woody materials are used more steadily than other natural sources because their carbon is not rapidly depleted [17]. In this way, they do not require frequent replenishment from the biological reactors.

3.2 Leaching of nutrients to the aqueous media. The release of TKN during the tests is shown in Figure 3. After 50 days, barley grains yielded the highest concentration among the materials tested, of about 5 mg N/L. This can be attributed to the protein content of this cereal (7.5 - 15.6%) [28], which is thereafter released into water. The concentrations of TKN released by woodchips (0.6 mg N/L) and peanut shells (0.74 mg N/L) are considerably lower than that produced by barley grains. Actually, the former materials are composed mainly of organic molecules lacking of nitrogen, such as carbohydrates; this was also indicated by the elemental analyses of the three materials.

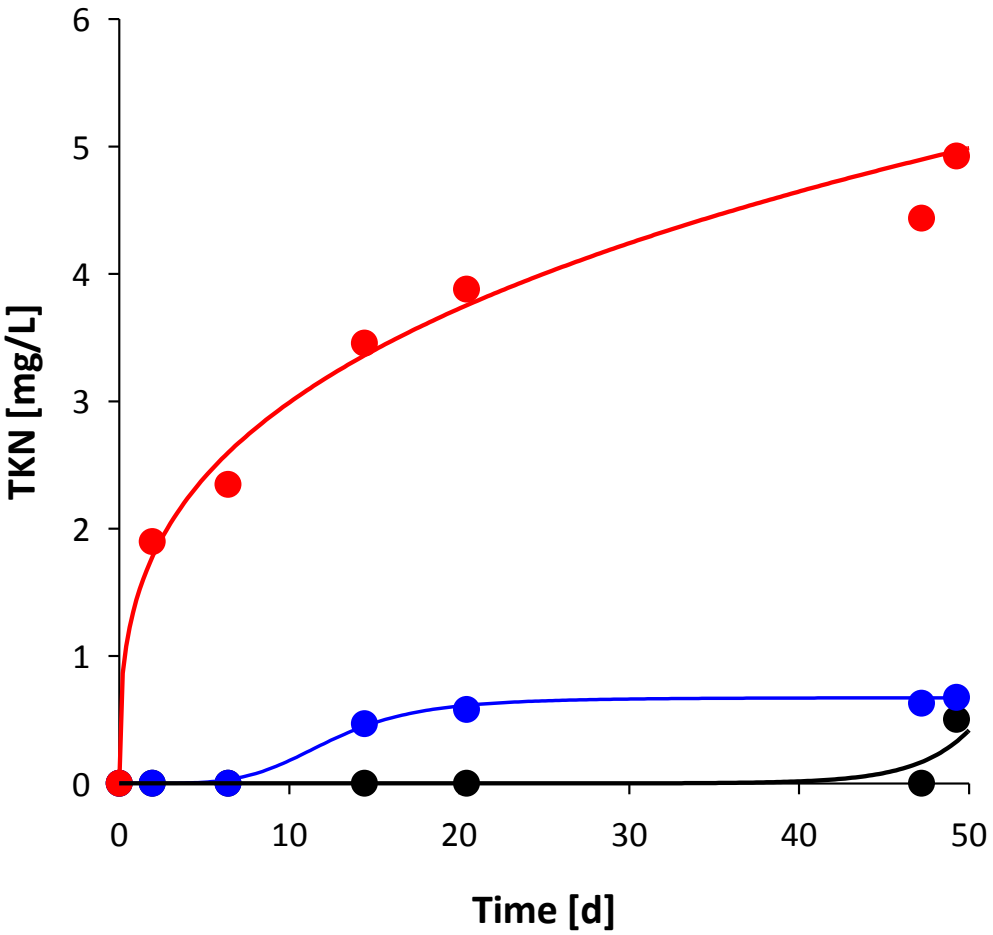

Figure 3. Leaching of total Kjeldhal nitrogen (TKN) from natural materials.

(●) Woodchips, (●) peanut shells, (●) barley.

Figure 4 presents the different nitrogen species leached from each material after 50 days. Any concentration of nitrites was detected in the eluates (the detection limit of the technique used is 0.04 mg $N\text{-}NO_2^-$/L). As mentioned before, barley yielded the highest concentrations of reduced nitrogen (i.e., organic and ammonium nitrogen) due to its protein content. The nitrates found in the barley leachate could arise from fertilizers applied to soil and after transferred to the plant, as suggested previusly by the FT-IR analysis.

Woodchips released the highest concentration of nitrates (3.4 mg N/L), followed by barley (2.2 mg N/L) and peanut shells (1.4 mg N/L). Since the elementary analysis indicated that woodchips does not present a measurable amount of organic nitrogen or ammonium, the nitrates leached might come from dirt or another type of foreign material.

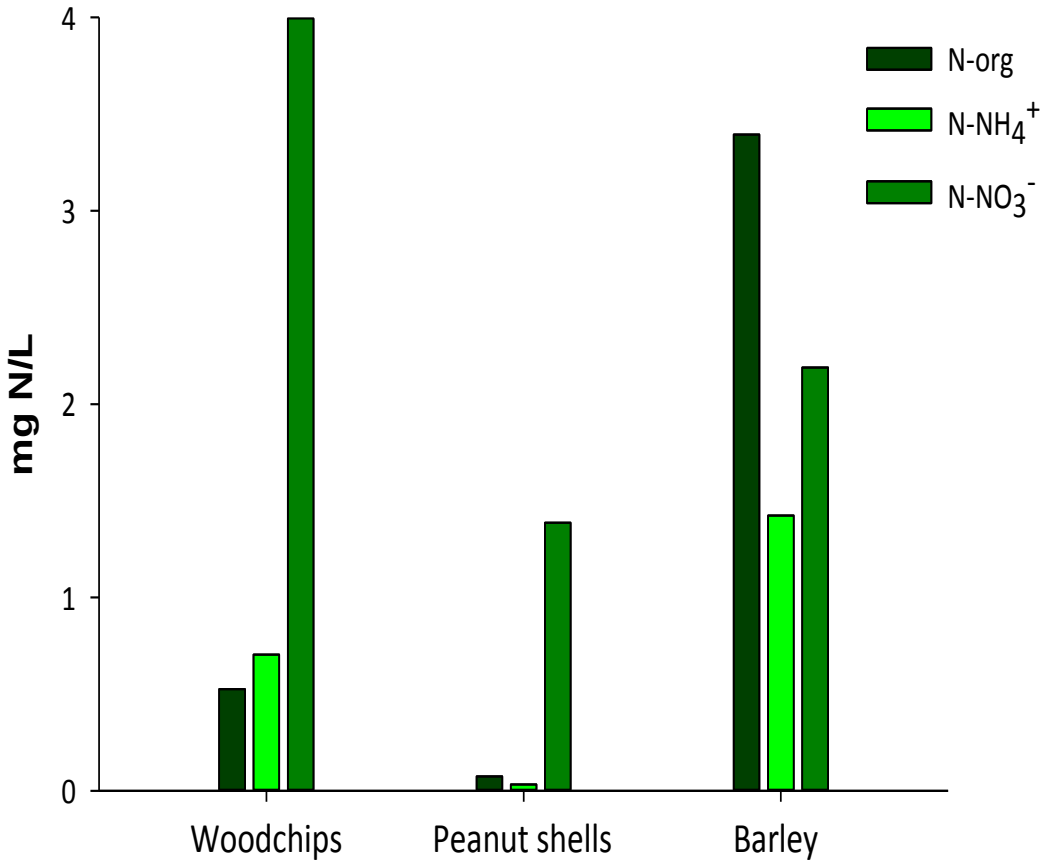

Figure 4. Nitrogenous species leached from natural materials after 50 days of testing

Peanut shells produced the least concentrations of nitrogenous species, due to the low content of protein of this material (6-7%) [29]. Peanut shells are composed mainly of fiber (60-67%)[29], and their content of lignin (32%) is even higher than those of most of hardwoods and softwoods [30]. However, peanut shells disintegrate easily in aqueous media, and produce high quantities of suspended solids (data not shown). Therefore, their use in biological reactors would require continuous replacement from the treatment system.

When all the nitrogenous species (i.e., TKN plus nitrates) are considered, barley is the material that releases the highest amount of this nutrient to the liquid media (Figure 5). At the end of the leaching tests, the materials had released 0.13, 0.04 and 0.31 mgN per gram of natural material. In the case of barley, for which an initial N content of 0.97% had been determined by elementary composition analysis, the release of N to the liquid media only corresponded to 3% of the initial input of this element.

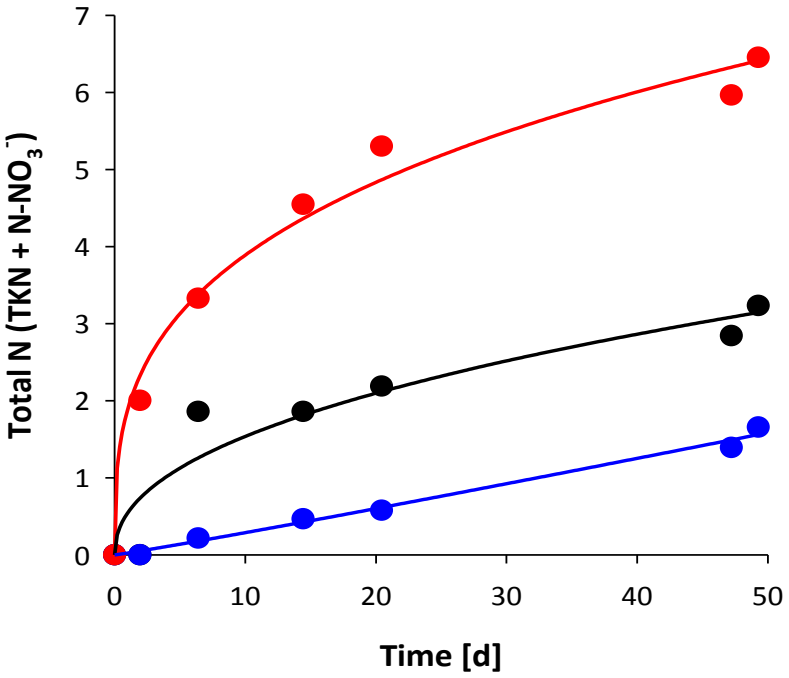

Figure 5. Leaching of total Kjeldhal nitrogen (TKN) from natural materials.

(●) Woodchips, (●) peanut shells, (●) barley.

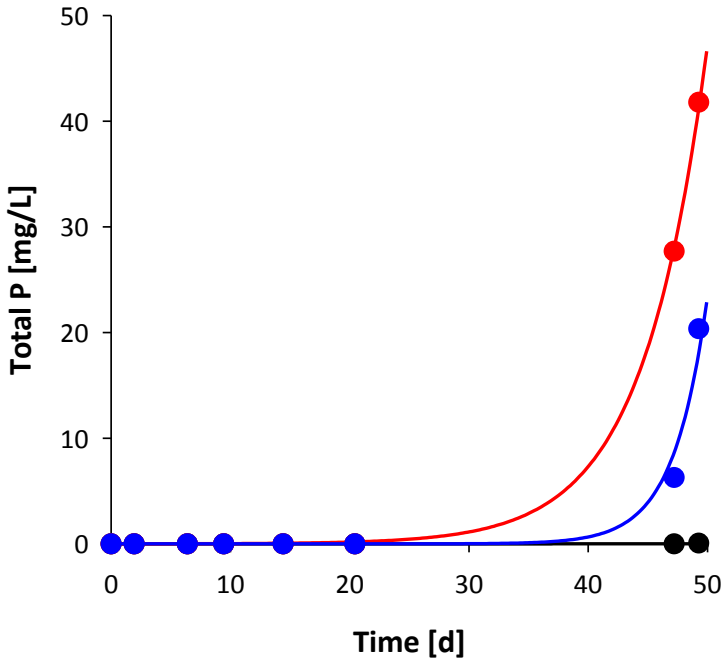

Figure 6. Leaching of total phosphorus (TP) from natural materials.

(●) Woodchips, (●) peanut shells, (●) barley.

The release of total phosphorus by the three materials is shown in Figure 6. Woodchips released barely detectable amounts of phosphorus. Again, the barley grains yielded the highest concentrations (superior to 40 mg/L). Similarly to nitrates, the leaching of this

nutrient from the grains may be due to the use of a combination of nitrogen and phosphate fertilizers in order to enhance the crop yield. These labile compounds can migrate easily from soils and then be retained by barley plants.

Table 2. Main characteristics of the FT-IR spectra of the lyophilized materials

Material	$\bar{\nu}$ **(cm^{-1})**	**Assignment**
Woodchips	3440	υ_{O-H}
	2925	υ_{C-H}
	1639	δ_{O-H}
	1378	$\upsilon_{N=O}$
	1033	υ_{P-O-C}
	755	υ_{P-C}
Peanut shells	3440	υ_{O-H}
	2957	υ_{C-H}
	2926	υ_{C-H}
	2854	υ_{C-H}
	1638	δ_{O-H}
	1401	$\upsilon_{N=O}$
	1033	υ_{P-O-C}
	834	υ_{C-H}
	756	υ_{P-C}
	702	υ_{P-C}
Barley	3424	υ_{O-H}
	2964	υ_{C-H}
	2928	υ_{C-H}
	2856	υ_{C-H}
	1631	δ_{O-H}
	1569	υ_{N-O}
	1404	$\upsilon_{N=O}$
	1062	υ_{P-O-C}
	758	υ_{P-C}

υ: Stretch vibration; δ: scissor vibration.

3.3 Chemical composition of the lyophilized leachates. The elemental composition analysis showed that woodchips yielded the leachate with the highest carbon percentage (50. 4%), followed by barley (26.7%) and peanut shells (21.7%). Concerning the nitrogen content, barley leachate presented the highest percentage (1.9%). Peanut shells released an eluate with 1.5% of nitrogen, while in the leachate from woodchips this nutrient was not detected.

The main band assignments of the FT-IR spectra of the lyophilized leachates are presented in Table 2. They are very similar to those found for the raw natural materials. In the leachates, the bands corresponding to the O-H stretching vibrations were also observed in the ≈3400 cm^{-1} region, as well as C-H stretching bands (≈2900 y ≈2850 cm^{-1}) associated to common organic compounds as carbohydrates and lipids. The presence of inorganic compounds as

phosphorus in the leachates of the three materials is confirmed, because oscillating signals in the 750-1000 cm^{-1} region were detected. As stated above, this could be explained by the addition of phosphorus fertilizers to soils.

The signals detected at 1569 and 1404 cm^{-1} in the barley spectrum are related to nitrates, which could be explained by the use and plant accumulation of fertilizers. According to Kumar and Goh [23], ammonia-based fertilizers might also contribute to the plant nitrate content, since ground nitrifying bacteria oxidize NH_4^+ to NO_3^-. These labile ions can easily pass from the soil to the plants and then to the aqueous leachates. It is worth to note that the presence of this functional group cannot be linked to the natural protein content of the grain, because the bands assigned to these organic compounds appear in the 3400-2900 cm^{-1} region due to the high carbon concentration compared to the nitrogen content. These results demonstrate that barley is not well-suited as carbon donor, because it can be a source of cross-pollution due to nitrogen and phosphorus compounds.

4. Conclusions

We evaluated three natural materials –woodchips, peanut shells and barley grains– as potential carbon donors for the biological treatment of wastewater with an unfavorably low carbon-to-nitrogen ratio. Although the elemental analysis indicated that only barley could release nitrogenous compounds to aqueous media, the analyses of the leachates demonstrated the transfer of nitrates from woodchips and peanut shells to the corresponding eluates. This was confirmed by the FT-IR spectroscopy analysis of the raw materials and of the lyophilized leachates. The FT-IR spectra revealed also the presence of phosphate groups in the raw materials and in the lyophilized leachates.

The results of the leaching tests indicated that woodchips and peanut shells are suitable carbon sources, as they release organic matter but lesser amounts of nutrients than barley. Organics produced by woodchips are less easily biodegradable than those released by peanut shells. This implies that woodchips donate carbon in a steady way, and that they do not need to be replenished frequently from the biological reactors. By contrast, peanut shells disintegrate easily, thereby increasing the content of suspended solids.

In conclusion, woodchips may be considered as an adequate and economical carbon donor that minimizes cross-pollution in wastewater treatment.

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Capítulo 8
Conclusiones

La caracterización de los efluentes de la Granja Integral de Policultivo indicó que el tratamiento biológico es el más factible para cumplir con los requisitos que establecen los sistemas de recirculación para la acuacultura.

Un proceso de biofiltración en dos etapas (nitrificación/desnitrificación) con CAG como material filtrante es una alternativa favorable para el medio acuático, ya que permite la eliminación de SST, DBO_5 y NTK de los efluentes acuícolas. Este proceso también debería asegurar la eliminación de los contaminantes microbiológicos encontrados (coliformes fecales, *Salmonella spp.*, *Staphylococcus aureus* y *Vibrio cholerae*) con el fin de evitar la contaminación de los peces cultivados.

La eliminación completa de nitrógeno implica el uso de microorganismos heterótrofos, que lleven a cabo la reducción desasimilatoria de nitratos que a su vez está acoplada a la oxidación de materia orgánica. Sin embargo, los niveles de COT, DQO y DBO_5 medidos en el efluente señalan que el proceso de desnitrificación requiere la adición de un material orgánico como fuente exógena de carbono.

El CAG mostró una capacidad de adsorción menor a la reportada en la bibliografía para el azul de metileno en las pruebas por lote (estáticas y dinámicas). Sin embargo, en pruebas continuas en una columna alimentada con el efluente acuícola y empacada con CAG, disminuyeron significativamente los contenidos de $N\text{-}NH_4^+$, P-total y coliformes totales.

Los resultados de la evaluación de los materiales indicaron que el aserrín y la cáscara de cacahuate son fuentes adecuadas de carbono, en la medida en que liberan materia orgánica suficiente y una cantidad limitada de nutrientes. En particular, el aserrín libera carbono de manera constante y por lo tanto no necesita ser reemplazado con frecuencia de los reactores biológicos; además, aporta bajas concentraciones de especies nitrogenadas y cantidades no detectadas de fósforo.

Capítulo 9

Perspectivas

Uno de los resultados de este trabajo fue determinar que una columna de adsorción empacada con CAG permite disminuir de modo importante las concentraciones de los contaminantes producidos por la industria acuícola. Sin embargo, para conocer los alcances de esta operación, aún es necesario determinar el área superficial del CAG y evaluar la capacidad de adsorción de este material a diferentes tiempos de retención de lecho vacío (TRLV). De esta manera, será posible optimizar el TRLV y el nivel de tratamiento alcanzado.

Por otra parte, también es necesario obtener consorcios nitritantes, nitratantes y desnitrificantes a partir de aislamientos sucesivos de muestras de agua superficial en medios formulados para el desarrollo de tales microorganismos. Luego de verificar su actividad en pruebas cinéticas, estas cepas se usarán para formar biopelículas adheridas al CAG. Además, en el caso de las bacterias desnitrificantes, deberá verificarse que estas efectivamente utilicen el carbono lixiviado por el aserrín. Ambos biofiltros deberán acoplarse para que el efluente del biofiltro nitrificante se trate en el biofltro desnitrificante. Por último, el funcionamiento de los biofiltros deberá validarse con el efluente acuícola de la Granja Integral de Policultivo de Tezontepec de Aldama, Hgo.